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Evaluation of in situ bioremediation approaches in meeting international standards for organic and residual metals toxicity in soils

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EVALUATION OF *IN SITU* BIOREMEDIATION APPROACHES IN MEETING INTERNATIONAL STANDARDS FOR ORGANIC AND RESIDUAL METALS TOXICITY IN SOILS

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ABSTRACT

Drilling fluids and muds are an essential component of the rotary drilling process used to drill for oil and gas on land and in offshore environments. However, the bioaccumulation of petroleum contaminated soils/drilling mud due to oil and natural gas exploration has posed a major environmental concern due to its prolonged environmental persistence and its leachability below the rhizosphere. The main objectives of this research were: (i) To assess initial toxicity of drilling mud and related contaminated soils from an agricultural setting in Galliano, Sicily (Phase one) and (ii) To develop a low intervention bioremediation approach using bioplug technology to ensure that the soil/drilling mud has met U.S. and international soil/ground water quality standards by performing a microcosm study (Phase two). Total petroleum hydrocarbon content of the drilling mud reduced to 617.0±176.0, 446.0±195.0, and 533.0±138.0 mg/kg from 5000.0±530.0 mg/kg after treatment via mixing (84.2-95% reduction). The PAH and phenol concentration of the drilling mud resulted in a 97-99.5% reduction via mixing (5d study) and 69.4-77.9% reduction via *in situ* treatment (40d study). The metals of concern for the drilling mud are cadmium and selenium. Both metals had exceeded Italian and La DEQ soil leachability standards, which were established at 0.005 mg/L for cadmium and 0.010 and 0.050 mg/L for selenium, respectively. *In situ* bioremediation was performed on a cross-section of Italian soil/mud to test the effectiveness of bioplug technology. Total petroleum hydrocarbons had reduced from 217.12±43.38 and 149.68±45.51 mg/kg to 15.16±3.35 and 34.27±15.86 mg/kg for the control drilling mud test beds, and from 89.20±67.42, 141.71±64.80, and 197.87±77.38 mg/kg to 5.24±6.15, 15.02±10.20, and 9.65±9.37 mg/kg for the experimental drilling mud test beds, respectively. The

efficiency of degradation for control and experimental setups were 85.1±11.2% and 92.9±3.0%, respectively. Overall, the microcosm experiment indicated that a significant reduction in total petroleum hydrocarbons had taken place for the drilling mud using bioplug technology and will be installed at the Italian site.

1. INTRODUCTION

Over the past several decades, man has grown more and more reliant on fossil fuels. Many of the comforts that man enjoys today are possible largely because of fossil fuels such as petroleum. This increase in use has resulted not only in an increased dependence but an increase in petroleum related pollution.

The need to remediate contaminated sites is essential for protecting human health and environmental ecosystems. Bioremediation, the use of microorganisms to degrade contamination to less toxic substances, is being used more frequently as a treatment option. Bioremediation has the advantage of cleaning up wastes *in situ* and generally at lower cost. Aerobic biodegradation involves microorganisms that degrade oil or other organic contaminants, which then digest the oil and convert it to carbon dioxide (CO_2) and water (H_2O), and then they give off CO_2 and H_2O. Immobilized bed bioreactors, or bioplugs, can be used in the bioremediation process to help ensure rapid degradation. Bioplugs are packed bed reactors strategically placed in contaminated areas to generate sufficient biomass for petroleum hydrocarbon degradation. These bioplugs also have the advantage of treating contamination in place without impeding the demolition of surrounding structures. This technology may prove useful on industrial sites that require a fast and effective remediation alternative for cleaning petroleum contamination.

An agricultural setting located in Galliano, Sicily contains drilling mud and related contaminated soils from natural gas exploration. The Italian government is interested in evaluating a low intervention bioremediation approach for biological treatment of drilling mud and related contaminated soils at their site of origin. Soil samples from Galliano and related contaminated soils from Syracuse, Sicily were sent to

Louisiana State University-Dep't. of Environmental Studies (LSU-DES) to determine efficiency of biological treatment and to determine whether an *in situ* bioremediation system will be feasible for the cleanup of the site.

The focus of this research project was to: (i) Evaluate a strategy to minimize the need for excavation and offsite transport/disposal of site soils, (ii) To develop a biological treatment system, which can treat site soils at their point of origin and (iii) To prevent the further leaching of contaminated soils to underground water sources. In order to determine the effectiveness of this strategy, a two-phase process was utilized. A lab treatability study, Phase one, was first performed that consisted of (i) Initial screening of the site soil and drilling mud using various analytical techniques and (ii) A 5 d bioreactor experiment to assess efficiency of biological degradation. Phase two consisted of a microcosm study, which was constructed in the laboratory. The goals of phase two were to: (i) Simulate a cross-section of the Italian landscape on an inclined plane, (ii) The design of PVC piping for the aeration, biological treatment, and saturation of the site soils, (iii) Determine chemical properties of site soils and drilling mud during biological treatment, (iv) To meet U.S. and international soil/ground water standards and (v) To assess whether construction of the *in situ* bioremediation system can be performed at the Italian site. If successful, this approach will provide a fast, effective, low intervention method for industrial site cleanup and can be utilized for contaminated sites in international regions.

2. LITERATURE REVIEW

2.1 Horizontal vs. Vertical Drilling: Oil/Natural Gas Production

Oil is a *complex mixture of varying quantitative composition*. Oils have the same molecular compounds/structures in them, but they are present in differing relative concentrations. Oil products are *complex mixtures with selective compounds refined out to produce products with desirable characteristics*. When a well is drilled, the oil migrates into a pipe through pumping action and then the oil is taken to a refinery. The refinery separates the oil from the highly saline water and discharges the water fraction. Oils contain many aliphatic and aromatic hydrocarbons which when present in any environmental system can cause a detrimental effect to many organisms and its habitat. Oil and natural gas exploration is largely found in many coastal/industrial regions and is a concern for many types of soils/drilling mud (Overton et al., 1994).

Since the first commercial oil well in 1859, the United States has produced somewhat more than 100 billion barrels of oil, most of it in recent years. In 1994, world petroleum consumption was at a rate of about 65 million barrels per day. Liquid petroleum is found in rock formations ranging in porosity from 10 to 30%. Up to half of the pore space is occupied by water. Natural gas, consisting almost entirely of methane, has become more attractive as an energy source with substantial new sources of this premium fuel. In addition to its use as a fuel, natural gas can be converted to many other hydrocarbon materials. Natural gas is an expensive commodity but can provide abundant energy reserves (Manahan, 2000).

There are many types of wells that can be drilled for oil/natural gas production. The major purpose of horizontal well drilling is to increase the contact area with reservoir

exposed in the well and thereby enhance well productivity. Horizontal well technology was first developed in China which consisted of more than 150 horizontal wells with various radii, most of which were located in Shengli field, have been drilled since 1990. Compared to a vertical well, a horizontal well is generally specific in its horizontal section, is less resistant to flow, has a large area of drainage, and has a longer period of contact with drilling muds. In addition, the fluid flow for a horizontal well is affected by the anisotropy of permeability. The oil production rates of most horizontal wells are three to five times higher than that of their adjacent vertical wells, which is highly related to the effective prevention of formation damage (Yan et al. 1998). The abundance of these fossil fuels has led to an increased dependence and petroleum related pollution on land and in offshore environments.

2.2 Toxicity of Drilling Mud

Drilling muds are modeled as a suspension of clay particles and high-gravity solids in water or oil, with the acoustic properties of these fluids depending on pressure and temperature. Since mud at different depths experiences different pressures and temperatures, downhole mud weights can be significantly different from those measured at the surface (Carcione and Poletto 2000). In their function, muds may be compared with cutting fluids in machine tools. The functions of mud consist of: (i) Pressure support of the drilled hole where its weight and consistency prevents ingress from the surrounding strata into the hole and has a plastering effect, preserving the integrity of the hole as drilling proceeds, (ii) The fluid mud circulates down to the drill bit and back to the surface, bringing with it the cuttings, and (iii) The mud cools and lubricates the drill bit and drill-string when working (Grieve 1988).

The specific type of drilling mud, which was utilized in this research, was an oil-based drilling mud. Oil-Based Muds (OBM's) are an emulsion of water in oil, hence the more correct name Invert Oil Emulsion Mud (IOEM) which contains approximately 10-50% water content, but typically shown to contain 30%. OBM's contain surfactants both as emulsifiers and wetting agents. OBM's have the same ingredients as Water-based Muds (WBM's), which includes (i) Water and clay where clay is added to provide viscosity and filtration control; typical clay used Bentonite, (ii) Barytes, iron oxides, and carbonates, (iii) Viscosifiers and fluid loss reducers, (iv) Deflocculants (thinners, dispersants), (v) Oxygen and hydrogen sulfide scavengers, (vi) Loss circulation materials (perlite), (vii) control pH, salinity (NaCl, KCl, NaOH, gypsum), and (viii) Lubricants, detergents, and defoamers (Grieve 1988).

The mud is formulated at ambient temperatures in volumes of up to 1000 barrels. It is ideally kept at a pH of around 10, which reduces bacterial contamination of the mud. The health effects, which are known to occur from drilling mud, are as follows: (i) Irritation of the skin, eyes, or alimentary mucosa can be caused by either low pH mud, surfactant or nuisance dust (De-aromatized hydrocarbons can have enhanced irritancy), (ii) Secondary irritation where prolonged and repeated contact (base oils/solvents) with skin will remove natural fats and oils and cause redness, drying and cracking, (iii) Respiratory irritation primarily from nuisance dusts, (iv) Inhalation effects such as acute CNS depression is an acknowledged effect of work with hydrocarbon solvents, especially at elevated temperatures. Solvents used in OBM's are of low vapor pressure, and thus should not cause problems of CNS depression, although nausea and headache can occur, (v) Ingestion is not a problem in normal usage, (vi) Biocides in muds may give rise to

sensitization, and (vii) Risks of PAH's and asbestos can give rise to carcinogenicity in muds (Grieve 1988).

2.3 Immobilized Bed Bioreactors (IMBR)

Microbial degradation is a naturally occurring process used to mineralize toxic organic pollutants (pesticides, petroleum products, amines, and other organic chemicals) into non-toxic metabolites, carbon dioxide, and water. Microorganisms can evolve to metabolize anthropogenic organic pollutants as their carbon source when these chemicals are introduced into their microenvironment (USEPA Feb. 1991; Portier et al. 1995). However, this process is usually impeded by limited supplies of inorganic nutrients and oxygen available in contaminated soils and groundwater. As a result, it can take many years to bioremediate organic pollutants from soils or groundwater using only natural attenuation (Smith and Portier 1997). Several methods have been designed to hasten the growth rate and transport of contaminant metabolizing microbial populations. Immobilized bed bioreactors (bioplugs) are one *in situ* method that facilitates the bioremediation of organic contaminants from soils and groundwater with minimal alteration to the environment (low intervention). By treating contaminants *in situ,* the problem is not only localized, but also any unnecessary transportation or digging costs are eliminated. Bioplugs are designed to place selected microorganisms in proximity to the contaminants of concern. An example of a typical bioplug is given in Figure 2.1.

Each bioplug is constructed using slotted PVC pipe or HDPE pipe that is sealed at each end. Each plug is filled with inert porous material, which serve as a support or attachment matrix for the microorganisms (Portier et al. 1995). Microorganisms are selected based on their predetermined ability to degrade the constituents of concern.

Often mixed consortiums of microorganisms are used in order to achieve optimum degradation potential (Smith and Portier 1997; Portier et al. 1995).

Once constructed, the bioplugs are ready for installation. Each bioplug is inserted in a strategic position in the zone of contamination. Liquids flow through the plugs by a pressure gradient created using compressed air. The purpose of this flow is to accelerate the flow of ground water through the immobilized beds, thereby facilitating the mineralization of organics within the aqueous phase and the bulk generation of biomass that bleeds off the bed. The microbial cells bleeding off the immobilized bed then exit the plug at the soil interface. Thus an enriched, adapted microbial population will percolate through the unsaturated zone. Each plug can also be connected to a tank where it can be fed a nutrient solution to assist in providing an optimum growth environment (Portier et al. 1995).

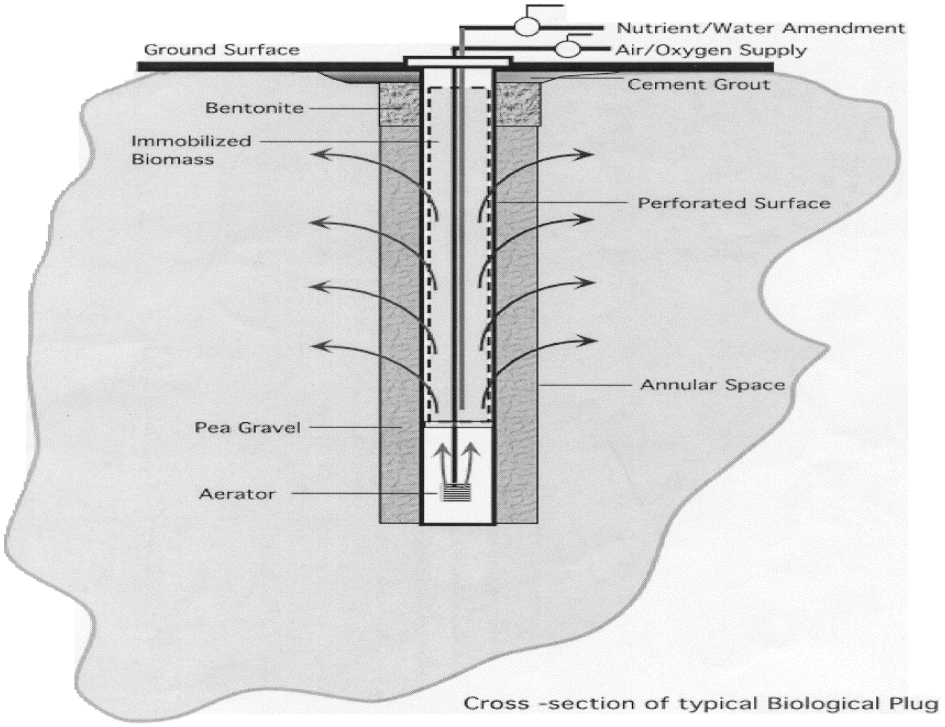

Figure 2.1: Cross-section of typical bioplug

Hydraulic control of the treatment area is quite important. The plugs will generate a hydraulic gradient at the site, either as a result of the injection of water through the bioplugs or the injection of air, which can change the pressure gradient. Hydraulic control generally consists of some method of collecting the water down gradient from the region of influence, i.e. a recovery trench, and recycling it back to the site through the bioplugs. There are two major reasons for recycling the water; (i) the recycling will control the migration of contaminants from the affected area and (ii) recycling of the water will help maintain the nutrient concentration in the injection water and re-introduce adapted microorganisms back into the affected area (Portier et al. 1995).

Another process in which petroleum hydrocarbons can be degraded is through bioventing. Bioventing is the process of supplying air or oxygen to the unsaturated zone to stimulate aerobic biodegradation of a contaminant. Bioventing is applicable to any contaminant that is biodegradable aerobically. Air can be injected through boreholes screened in the unsaturated zone, or air can be extracted from boreholes, pulling air from the surface into a contaminated area (Norris et al. 1994).

In general, *in situ* bioremediation costs are dependent on a number of factors including site conditions, remedial goals, the design of the system, and the operating and monitoring schedule such as: (i) Mass of contaminant, (ii) Volume of contaminated aquifer, (iii) Aquifer permeability/soil characteristics, (iv) Final remediation levels, (v) Depth to water, (vi) Monitoring requirements, (vii) Contaminant properties, and (viii) location of the site (Norris et al. 1994).

2.4 Heavy Metals in the Environment

Metals are present everywhere in the environment and are different from other toxicants because they are neither created nor destroyed. Metals incorporate in an organic compound or inorganic salts. Distribution in the environment occurs naturally via a geologic and a biological cycle (Klaassen, 2001). Heavy metals are toxic in their chemically combined forms (as with mercury) and some are toxic in the elemental form. The temperature, pH, and reducing nature (as expressed by the negative log of the electron activity), pE) of the solvent medium are important. The nature of the solids, especially the inorganic and organic chemical functional groups on the surface, the cation-exchange capacity, and the surface area of the solids largely determine attenuation of heavy metal ions. In addition to being sorbed and undergoing ion exchange with geospheric solids, heavy metals may undergo oxidation-reduction processes, precipitate as slightly soluble solids (especially sulfides) and in some cases, such as occurs with mercury, undergo microbial methylation reactions that produce mobile organometallic species (Manahan, 2000).

In some instances, a chelating agent may be necessary to break down the toxic effects of heavy metals. It is essential to break down heavy metals in the environment, especially in soils, to protect human health and to prevent bioaccumulation in ecosystems. Soil is susceptible to heavy metals in the environment due to its composition; it is a variable mixture of minerals, organic matter, and water. Soil formation is largely from the final product of weathering action of physical, chemical, and biological processes on rocks, which largely produces clay minerals. The organic fraction (humus) consists of plant biomass in various stages of decay (Manahan, 2000).

3. MATERIALS AND METHODS: SITE CHARACTERIZATION/APPROACH

3.1 Background of Italian Site

The landscape of the Galliano site consists of two impoundments filled with drilling mud and covered with a layer of topsoil. Each impoundment has a different bottom elevation, are separated by a retaining wall made up of rocks held together by a metallic mesh, and placed perpendicularly to the direction of the maximum slope. The construction of the retaining wall around the contaminated site has provided for the mitigation of earth slides, running water and rainfall. The composition of the topsoil present at the Galliano site consists of 75% clay, 13% lime, 10% sand, and 2% gravel, which its thickness in the impoundments covers approximately 1 to 2 m in depth. The drilling mud contains 96% clay, 2% lime, and 2% sand with a variable thickness of approximately 0.9 to 1.6 m. The underlying soil contains 80% clay, 10% lime, and 10% sand with a thickness of approximately 1 m (Verbal Communication, 2001). Some major considerations in the development of the system and proper treatment of the soil/drilling mud is to have an adequate water supply to be fed in the system, due to the fact that water percolates around clay particles (Manahan, 2000) and to have an abundant microbial population for degrading the organics in the mud/soil.

3.2 Objectives and Rationale

The primary objective was to determine the percent biodegradation of total TPH's ($TPH_{-kerosene}$) in the soil/sediments during the screening biotreatment study and to what extent metals are removed. The secondary objectives for the treatability study are to determine the percent biodegradation of critical organics and metals in the soil/sediments; to determine the mass of organics/metals transferred from the soil/sediments to the water;

to estimate the mass of volatile organics volatilized; and to determine the percent biodegradation of organic/metal intermediates in soil/sediments (Portier 1998). Hypothesis: Drilling muds can be remediated using an *in situ* approach.

3.3 Research Approach

3.3.1 Phase One Overview

A screening study on site soils was first performed to determine what the impact of implementing a bioplug remediation system at the Galliano site would be. Three containers were sent to LSU from Galliano, Sicily containing drilling mud and topsoil. The first set of analyses consisted of using EPA Standard Method 3550B ("Ultrasonic Extraction") to extract nonvolatile and semivolatile organics from the soil matrix and then using EPA Method 8270C for GC-MS to determine PAH and phenol content in the soil/drilling mud. The analyses helped determine which container of drilling mud/top soil was most contaminated. Assessment of total petroleum hydrocarbon content was performed using EPA Method 418.1, spectrophotometric analysis.

The initial screening of the drilling mud from Galliano, Sicily consisted of total petroleum hydrocarbon content, gas chromatography, density, specific gravity, microbial plate counts to determine biomass, pH, water leachability test, and a total dry phase. The biodegradation experiment for the drilling mud consisted of a clean container, where 1 kg of soil to 4 L of water was added. The soil was mixed via a rotary blade to homogenize and break down approximately 24 h before addition of the inoculum. Approximately, 100 ml of inoculum was used from a similar site in the U.S. Also, 0.5 g of K_2PO_4, 0.5 g KH_2PO_4, and 0.5 g of ammonium nitrate were added nutrients for microbial enrichment. The total length of the experiment lasted for 5 d. Treated soils/mud were allowed to

settle, screened for solids, and centrifuged to separate the supernate from the "cake" layer. Solid and liquid extractions were completed for control and treated materials to assess initial contaminant concentrations and efficiency of biodegradation. A comparative test using related contaminated soils from Syracuse, Sicily was also evaluated. The texture of the drilling mud from Galliano compared to the contaminated soils from Syracuse was different in composition.

3.3.2 Phase Two Overview

A recommended approach to developing a low intervention bioremediation system for the Galliano site is to dig a trench at a certain depth to reach the drilling mud, drill into the ground at a 2° slope, and insert the "bioplugs" horizontally across the width of the landscape. To test the technique, a microcosm experiment was performed using the samples obtained from Galliano.

The purpose of the microcosm setup was to build in the laboratory a field of the Italian site. The microcosm setup consisted of three experimental and two control bed reactors with dimensions of 22.9"×17"×7.3", which contained ~1/4-1/2" of a sand base, ~1-2" of drilling mud and ~1-2" of topsoil. A random setup of the microcosms was designed in the laboratory. The positioning/labeling of the microcosms were as follows from left to right: Experimental I, Experimental II, Control I, Experimental III, Control II. The design of a bioplug consists of 1/8" perforated PVC piping 1" in diameter containing an air hose (porous tubing) and a biocarrier. Adapted microflora on a controlled porosity biocarrier, exit out the PVC piping with the flow of the site water. The bioplugs were inserted horizontally across the width of the containers, connected to an ell where vertically a 1/2" PVC piping facilitated the air/water supply, was connected to another

ell, tee, and a 1/2" valve, the air line connected to a manifold system to control air pressure and the waterline connected to an aspirator bottle, percolated by gravity. The setup was designed on an inclined plane for the collection of runoff. The runoff, based upon actual field measurements, was infiltrated back into the reactor, acting as a continuous flow-through system.

The variables monitored during the course of the experiment were: i) Environmental fate and transport of hydrocarbons, metals, and organics, i.e., a material balance, ii) Whether the drilling mud percolated into the sand layer, iii) Layer thickness of drilling mud that could be remediated using this technique, iv) Reduction in volume of the drilling mud/soil, v) Tracking migration of the drilling mud, and vi) Tracking the efficiency of the experiment by creating a grid, randomly sample, and obtaining a soil profile.

Triplicate soil samples were obtained where each were randomly chosen from five different locations of the container due to its heterogeneity to create a composite sample. The soil samples were collected at days 0, 7, 14, 28, and 40. Twenty grams of soil/mud was collected for each sampling jar for extraction and 10 g was obtained for a dry weight. The pH of the recycled water was monitored once a week. Microbial bleed off (biological activity) was determined by using a HYcheck^{TM} dipstick. The dipstick was immersed into 100 ml of sample, which was then preserved in an incubator for 3 d at 37ºC before enumeration. Nutrient levels were determined by testing for ammonia, nitrate, and phosphate levels using a CHEMet kit (colorimetric determination). If nutrient levels were low, 2 g/L of ammonium nitrate and 0.2 g/L of KH_2PO_4 monobasic were added to control pH levels and to ensure continuous microbial activity. Efficiency

of degradation of the site soils/mud was determined by performing ultrasonic extractions and by preparing the samples for GC-FID and GC-MS for PAH and phenol assessment. A total of thirty soil samples were collected at each time interval. A Microtox assay was performed to determine the fate of toxicity of the recycled water. A metals analysis was performed of the topsoil, drilling mud, and sand layer to determine whether induced microbial action degrades heavy metal complexes such as chromium, mercury, and cadmium. Figure 3.1 shows the experimental design for phase two, simulating an *in situ* bioremediation system installation. Each setup had a piece of aluminum foil to cover the topsoil from evaporative loss.

Figure 3.1: Phase two experimental design: Microcosm study

4. MATERIALS AND METHODS: SAMPLING/ANALYTICAL METHODS

4.1 Ultrasonic Soil Extraction

4.1.1 Approach

The soil samples were extracted using a modified version of EPA Standard Method SW-846 3550B ("Ultrasonic Extraction"). The ultrasonic process ensures intimate contact of the sample matrix with the extraction solvent.

4.1.2 Sample Preparation

The extraction procedure was conducted in triplicate in order to obtain a statistical average. The extraction was conducted by weighing out 10 g of soil on small aluminum trays and placed in an oven to dry overnight. The purpose of obtaining a dry-weight of the soil/drilling mud was to determine the water content in the samples and the value was needed to assess contaminant concentrations through GC-MS. Approximately 10 to 30 g of contaminated soil were weighed out in a 40 ml I-Chem bottle and approximately 2 to 5 g of sodium sulfate, which serves as a drying agent to eliminate some of the moisture present in the soil. Immediately prior to extraction a 1 ml aliquot of 40 mg/L 8270 surrogate standard was added to each I-Chem vial with a 1 ml glass pipette. The surrogate contains six compounds: phenol, 2-florophenol, 2,4,6-tribromophenol, nitrobenzene, 2-florobiphenyl, and p-ter-phenyl. The purpose of adding surrogate was to determine the extraction efficiency. Surrogate recovery was evaluated for acceptance by determining whether the measured concentration falls within the acceptable limits.

4.1.3 Dichloromethane (DCM) Extraction

Twenty milliliters of dichloromethane (DCM) was added to each sample, placed in an L&R Transistor/Ultrasonicator T-14B as a source of ultrasonic energy, and the

timer was set for 12 min. At the end of 12 min., the liquid layer was poured through a funnel lined with Whatman #2 150 mm diameter filter filled with Na_2SO_4 into a flat bottom flask, and rinsed residual sample with DCM. The sonication procedure was repeated two more times. Upon completion, the extracts were boiled down to 1 ml in a Büchi RE 111 Rotavapor, which were spun in a Büchi 461 Heated Water Bath for approximately 5 min. The samples were pipeted into 4 ml glass vials with screw top cap. If more than 1 ml of sample remained, it was blown down to the exact volume with nitrogen gas (95% purity). The samples were wrapped with Teflon seal tape, and refrigerated until analysis.

4.2 Total Soil Petroleum Hydrocarbons

4.2.1 Approach

A modified version of EPA Method 418.1 (spectrophotometric, infrared) was used for phase one to determine total petroleum hydrocarbon content (mg/kg dry soil weight) of the site soil/mud from Galliano and soil from Syracuse using an IR spectrophotometer (Model HC-404, BUCK Scientific, Inc). Extractable petroleum hydrocarbons are largely determined on industrial and domestic wastes. However, this protocol is currently being phased out due to regulated control of Freon usage and the rising cost of the solvent. EPA Standard Method 8015B ("Nonhalogenated Organics using Gas Chromatography/ Flame Ionization Detection") was used for phase two as an alternate method for TPH analysis. The purpose of obtaining favorable results for TPH reduction and microbial growth on soils extracted from the contaminated site was to determine if this type of remediation system would be effective for use at the Galliano site. Total petroleum hydrocarbons refer to a broad range of compounds including

aromatic (ring structure) and straight chain organic compounds.

4.2.2 Sample Preparation

Ten-gram samples of soil were removed from each sample jar and placed into 40 ml I-Chem bottles. Approximately three grams each of sodium sulfate and silica gel were added to each vial to dehydrate the soils and eliminate polar compounds, respectively. The contents of each vial were thoroughly mixed together with a metal spatula taking special care to break up any large chunks of soil.

4.2.3 Freon Extraction

To each vial, 20 ml of Freon was added. The samples were then thoroughly mixed on a vortex for 2 min. and then allowed to settle for 10 min. to let the layers separate. The liquid portion of each sample was filtered through Whatman number 1 filter paper in a glass funnel, taken care not to let any solids through, into a 25 ml volumetric flask, that was then stoppered. This process was repeated again. Both the filter paper and the funnel were rinsed with Freon. Enough Freon was added to the flask to bring the extraction level up to 25 ml.

4.2.4 Total Petroleum Hydrocarbon (TPH) Analysis

Total petroleum hydrocarbon concentrations were determined by procedures set forth by the manufacturer of the TPH analyzer (Buck Scientific). The instrument is turned on and allowed to warm up for 30 min. with the sample door open. In absorbance (ABS) mode, the display is adjusted to read 0.000. In the %T mode the %CAL is adjusted to read 100.00. The light beam is blocked in order to adjust the %T control to 00.00. The mode is then changed back to ABS and a Quartz cell filled with Freon is inserted into the instrument and its' absorbance read. If the Freon measures anything but

0.00, the instrument must be recalibrated so the absorbance will read 0.00. Standard test oil solutions of 12.5, 25, 50, 100, and 200 mg/L were prepared according to EPA Method 418.1 using an oil standard diluted with Freon. The absorbance of each standard was recorded and a calibration curve created. The absorbance of each soil sample was then measured. If any sample produced an absorbance outside of the calibration curve, then the sample was diluted with Freon and measured again until the absorbance fell within the appropriate range (Buck Scientific, 1993). The TPH concentration in the extracted sample was interpolated from the standard curve to find a concentration. The concentration in the test solution was interpolated from the standard curve and correlated back to the concentration in the original soil sample using the preparation and corresponding dilution factor:

$$\frac{\textbf{Interpolated concentration} \times \textbf{25ml sample}}{\textbf{grams of Soil}}$$

4.3 Total Heterotrophic Microorganisms in Soil Samples

4.3.1 Approach

The purpose of this technique was to assess the level of microbial activity of the soil/drilling mud before and after biological treatment (breakdown of PAH's and phenols). The total number of cultivable heterotrophic microorganisms in a soil sample can be obtained by plating a dilution of a sample in an appropriate culture medium. Nutrient Agar (NA) is a commonly used medium for isolating and cultivating a variety of fastidious microorganisms. This media was used as a quality control measure: an absence of growth on NA indicated an improper plating technique.

4.3.2 Sample Preparation and Plating

One gram of room temperature soil was diluted into 99 ml of sterile physiological saline in a 200 ml dilution bottle, resulting in a 10^{-2} dilution. Two drops of sterile tween 80 were also added to the solution in order to break up any chunks of soil. The sample was then shaken vigorously, and a subsequent dilution was made by taking 1 ml of the 10^{-2} dilution and placing it into a separate 99 ml container of saline, resulting in a 10^{-4} dilution. Serial dilutions were made and replicate plates were poured in the following manner: 1 ml out of the 10^{-2} and 10^{-4} dilutions and 0.1 ml out of the 10^{-3} and 10^{-5} dilutions. An illustration of this procedure is provided in Figure 4.1. The plates were incubated for 3 d in a 31ºC incubator.

4.3.3 Enumeration

Plates were counted following Standard Method 37-6.2.1 (Page et al, 1982, p.790), counting only those plates containing less than 300 colony-forming units (CFU's) per plate.

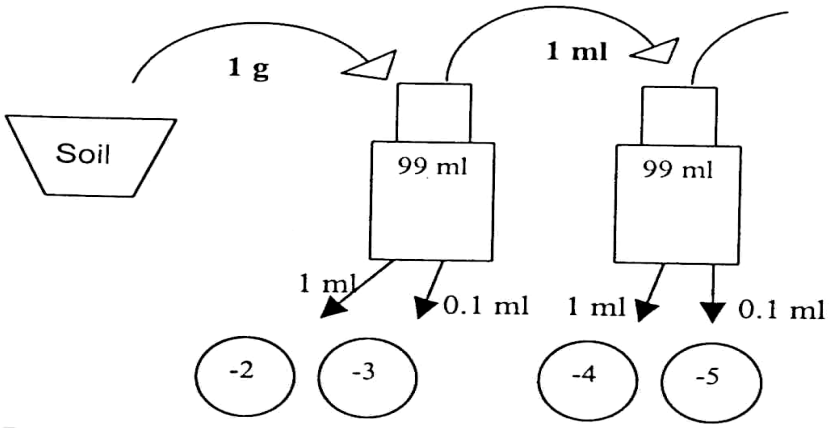

Figure 4.1: Illustration of soil sampling plating procedure

4.3.4 Leachable Fraction Biomass Enumeration

A HYcheck^{TM} dipstick (DIFCO, Report #L005259) was used for the monitoring of microbial bleed off into the effluent (Phase two). HYcheck^{TM} is a double-sided, hinged plastic paddle containing two agar surfaces. The agar surface extends above the paddle allowing for contact with test surfaces. The hinged paddle allows the agar surface to be easily held against each test area during sampling. The surface area of the paddle is clearly divided into seven units of one centimeter each to allow direct counting of microbial density per unit area. HYcheck^{TM} is an aid in assessing different types of microbial contamination. The dipstick has to be completely immersed in the sample in order to assure microbial growth on the agar. For the recycled water (Phase two), approximately 100 ml of sample from each microcosm was utilized to completely immerse the dipstick. Before a total count of colonies are assessed, the samples are incubated in an upright position at 35-37ºC examining within 2-3 d incubation. Both paddle sides have Plate Count Agar surfaces but one agar also incorporates 0.01% Triphenyl Tetrazolium Chloride (TTC). This aids the recognition of microorganism colonies due to the formation of the insoluble red formazan dye when the TTC is reduced. The growth of bacterial colonies in the sample is compared to a chart, which contains different growth patterns, and then an interpolation is made, recorded as bacterial count per mL (Becton Dickinson Microbiology Systems, 1999).

4.4 Gas Chromatography/Flame Ionization Detection

4.4.1 Approach

The flame ionization detector was adequate to perform a total petroleum hydrocarbon analysis and was an alternative method to EPA Method 418.1 by Freon

extraction. EPA Method 8015B was used to quantitatively determine the presence and concentration of PAH's and phenols. In summary, polynuclear aromatic hydrocarbons and saturated alkane content in soils contaminated with petroleum are determined qualitatively by extracting small quantities of soil with dichloromethane and injecting 1 µl of this extract into a gas chromatograph (GC) which contains a separation column specific for the desired compounds. A separation of different PAH components will occur as the sample is carried through a PAH specific column at constantly increasing temperatures. Separated compounds in the gas effluent are detected using a flame ionization detector (FID), which detects changes in an electric field as these compounds pass through a flame and electrons are exited (Blackman, 2001).

4.4.2 Extraction Procedure

The samples were prepared and extracted using a modified version of EPA Method 3550B, Ultrasonic Extraction.

4.4.3 Soil Analysis Using GC-FID

Analysis of samples were performed on a Hewlett Packard Model 5890 Series 1 gas chromatograph equipped with a 7673 autosampler and flame ionization detector. The column was a 30 m length (J & W Scientific DB-5) capillary column with an internal diameter of 0.25 mm, and 0.25 *u*m thickness containing 95% methyl and 5% phenyl stationary phase. Column capacity is approximately 250-300 mg/L. The injector and detector temperatures were set at 250ºC and 280ºC, respectively. Using temperature programming, the compounds could be eluted from the column separately. An initial oven temperature of 50ºC was held for 2 min. The temperature was increased 20ºC/ min

for a period of 12.5 min to a final temperature of 300ºC. The final temperature was held for 21 min, bringing the total run time to 35.5 min (Blackman, 2001).

One microliter each of a standard solution containing known concentrations of 2500, 1250, 625, 312.5, and 156.25 mg/L PAH's and phenols were analyzed to obtain reference peaks and retention times. From this data, samples could be analyzed and the PAH and phenol content quantified. A calibration factor was calculated based on peak height relative to concentration. The calibration factor will be used to calculate total petroleum hydrocarbon concentrations for sample extracts. After linearity of the standard curve has been confirmed, the samples can be analyzed. The auto sampler injected a 2 ul volume of each sample. Dichloromethane solvent blanks were placed at the beginning of the run and between groups of sample vials. All of the samples were quantified using the external standard procedure of a modified Method SW-846 8015B. The data generated was used to determine the levels of hydrocarbons that existed in the soil (Blackman, 2001).

4.4.4 Calculations

Calibration factors must be calculated for each of the standards and an average calibration factor must be computed using Standard Method SW-846 8015B:

$$\text{Calibration Factor (CF)} = \frac{A_s}{C_s}$$

Where:

A_s = Standard peak area

C_s = Standard concentration

Using the average of the calibration factors, the concentration of hydrocarbon compounds can be calculated in a sample using Standard Method SW-846 8015B:

$$\text{Concentration} = \frac{A_s \times V_t \times DF}{CF \times V_i \times W_e}$$

Where:

A_s = Sample peak area

V_t = Total volume of extract

DF = Dilution Factor

CF = Average calibration factor from standards

V_i = Volume of extract injected

W_e = Weight of soil extracted

4.5 Gas Chromatography/Mass Spectroscopy

4.5.1 Approach

Soil samples were analyzed using gas chromatography/mass spectroscopy (GC-MS) to determine possible breakdown products of PAH's and phenols. These samples were extracted using a modified version of EPA Method 3550B, Ultrasonic Extraction.

4.5.2 Operating Principles of a Gas Chromatograph

Chromatography is the general term for a separation method in which a mixture of components in a mobile phase passes through a stationary phase allowing components to partition out. In the case of gas chromatography, the mobile phase is a carrier gas and the stationary phase is generally a liquid sorbed on the surface of a solid. The degree of affinity for the stationary phase determines the length of time each component will remain on the column (Blackman, 2001).

Using a syringe, a liquid or gas sample is introduced into the injection port. A liquid sample will vaporize instantaneously and be carried through the column with an appropriate carrier gas. There are two general types of columns. The packed column is

filled with solid packing material coated with a large molecular weight grease-like compound. The capillary column has a much smaller internal diameter and is generally much longer than a packed column. The stationary phase of a capillary column also consists of a high molecular weight compound coated on the interior walls of the column. The column is located inside an oven, so that column temperature can be controlled (Blackman, 2001).

The retention time of a compound refers to the amount of time required for that compound to pass from the injector to the detector. The principle goal of gas chromatography is that each compound has a slightly different retention time, so separation, and ultimately identification can be achieved. In order for this to be accomplished, retention times can be manipulated using temperature programming, a method of ramping temperature at different rates to achieve better resolution between compounds and to faster elute peaks with long retention times. It is also important to note that retention time is dependant on several factors, including carrier gas flow, column temperature, affinity for the stationary phase, and length of the column. With the appropriate settings and temperature program in place, a sample can be successfully separated into its subsequent compounds before passing through the detector. The gas chromatograph can be paired with a number of different detectors, depending on the nature of the screening (Blackman, 2001).

4.5.3 Soil Analysis Using GC-MS

The samples were analyzed using a Gas Chromatograph (HP model #5890A) coupled with a Mass Selective Detector (HP 5970 Series). Prior to using the GC/MS, a standard autotune was performed in order to insure that the instrument is functioning

according to operating protocols. A 1ul aliquot of sample is injected on the column using an autosampler (HP 6890 Series). A high-resolution capillary column (J & W Scientific DB-5), measuring 30 m in length, with an internal diameter of 0.246 mm and a film thickness of 0.25 um was used. The injector and detector temperatures were set at 250ºC and 280ºC, respectively. An initial oven temperature of 55ºC was held for 3 min. The temperature was then ramped 5ºC/min for 45 min to a temperature of 280ºC. The temperature was immediately ramped again at 1.2ºC/min for 16.67 min to a final temperature of 300ºC, and a total run time of 64.67 min. In order to achieve a lower detection limit, the detector was placed in selective ion monitoring (SIM) mode. Prior to the analysis of samples, a standard curve was constructed by injecting calibration standards containing the following compounds: Phenols Mix, PAH Mix, Carbazole, Dibenzofuran, 2-methylnapthalene, and 8270 Surrogate Standard. From a prepared stock solution of 100ug/ml, concentrations of 5, 10, 15, 20, and 25ug/ml were prepared. The 1 ml volume of each standard was spiked with 10 ul of internal standard before injection on the column. From the standards, a response factor can be calculated for each compound using peak area relative to concentration. The response factor will be used to calculate the concentration of specific compounds of interest in each sample. After the linearity of the calibration curve is confirmed, samples can be analyzed with confidence. A solvent blank and one of the calibration standards are placed at the beginning of the sequence. Blanks are also placed intermittedly throughout the sequence. The auto sampler injects a 1ul aliquot of each sample. All samples were qualified and quantified using a modified SW-846 8270C. The data generated was used to determine which compounds are present and in what quantity they exist (Blackman, 2001).

4.5.4 Calculations

A response factor must be calculated for each of the compounds using Standard Method SW-846 8000B:

$$\text{Response Factor (RF)} = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

Where:

A_s = Peak area of analyte or surrogate

C_{is} = Concentration of internal standard

A_{is} = Peak area of internal standard

C_s = Concentration of analyte or surrogate

Using the Response Factor calculated for each compound, the concentration of individual compounds can be calculated with the following formula using Standard Method SW-846 8000B:

$$\text{Concentration} = \frac{A_s \times C_{is} \times V_i \times DF}{A_{is} \times RF \times W_e}$$

Where:

A_s = Sample peak area

C_{is} = Concentration of internal standard

V_i = Volume of extract injected

DF = Dilution factor

A_{is} = Internal standard peak area

RF = Response factor

W_e = Weight of soil extracted

4.6 Leachate Residual Analysis

4.6.1 Overview

The purpose of this technique was to determine what metals leach out from the solid to aqueous phase and whether a chelating agent was necessary to degrade heavy metals out of the bioremediation system. The water leachability test was a protocol developed by the Italian government. The leaching of the components was carried out by immersion of the sample in deionized water, which was changed at pre-established time intervals for total test duration of 14 d.

4.6.2 Experimental Setup

Upon experimental setup, a volume determination of the material to be analyzed was established, in liters, and its weight was recorded in kg. The reagents used for this experiment consisted of 1 M HNO_3 and deionized water. The utilized containers were closed to avoid exposure to the CO_2 in the atmosphere, which can cause pH variations. Two bioreactors containing a valve for the release of liquid, a neck for the renewal of water into the system, and a cover was clamped to seal the reactor. The containers to collect the samples, the bioreactors, and the filters to filter the samples were pre-rinsed with a 1 M solution of nitric acid for the purpose to remove any possible contaminant followed by rinsing with deionized water until every trace of nitric acid had been removed. The test was conducted at a temperature of 20±5ºC. The average temperature was recorded during each test interval. The weight ratio between the sample and the extracting solution had to have a value of five. The samples that underwent analysis were the drilling mud from Galliano, Sicily before and after biological treatment, which had a volume of approximately 200 ml. The samples were rolled into a spherical shape, tied

and wrapped in cheesecloth, and placed on the bottom of the reactor. One liter of deionized water was added to each bioreactor where the water was removed and replaced after 2, 8, 24, 48, 72, 102, 164, and 336 hrs for a total of 14 d.

4.6.3 Sample Collection

The containers to collect the samples were 16 media bottles each 500 ml, where 500 ml was utilized for metals and chemical oxygen demand and 500 ml for further analysis. Observational accounts of the liquid and solid phases were noted during replacement times. Upon completion, the samples were filtered through a 0.45 μm filter, pH readings were recorded and the samples were acidified with 1 ml of 1 M nitric acid solution. The samples were sent for analysis to determine what metals leachate out from the solid to aqueous phase (Methods 6010A and 7470A, U.S. EPA, 1983).

4.6.4 Digestion of Samples

Chemical oxygen demand was measured for aqueous samples and were assessed using Method 8000 for water, wastewater and seawater (Jirka and Carter, 1975). The purpose was to assure that the leachate of metals from the solid to aqueous phase did not exceed water quality standards. Before preparation of samples, the HACH COD reactor was turned on and preheated to 150ºC. COD Digestion Reagent Vials containing sulfuric acid were used for the digestion of the samples. Before selection of the vials, a range finding test was first conducted to determine the concentration range of the samples. The samples that underwent analysis were from the water leachability test. Upon completion of the test, 1500 mg/L sulfuric acid vials were selected for further analysis. The cap was removed from the vial, held at a 45˚ angle and 2 ml of sample was pipeted into the vial. The vial cap was tightly replaced, held by the cap, and inverted gently several times to

mix the contents. Special care was taken when mixing the contents due to heat generation. The vial was then placed in the preheated COD reactor. This process was repeated for all the samples. A blank was prepared by following the above procedure, substituting 2 ml of demineralized water for the sample. The vials were heated for a total of 2 hr. Upon completion, the reactor was turned off, waited 20 min. for the vials to cool to 120ºC or less, inverted each vial several times while still warm, and placed the vials into a rack for it to cool to room temperature.

4.6.5 Colorimetric Determination

The 16 samples from the water leachability test were analyzed by a HACH DR/2000 Direct Reading Spectrophotometer to assess the readings. A stored program number (# 435) for chemical oxygen demand was entered to the instrument. The wavelength dial was adjusted to 620 nm. Upon pressing the READ/ENTER button, the display showed mg/l COD H on the screen. The COD Vial Adapter was placed into the cell holder with the marker to the right. The outside of the blank vial was cleaned with a Kim-Wipe taking special care not to shake the vial's contents and gently placed into the adapter with the Hach logo facing to the front of the instrument. The cover was then placed on the adapter, pressed the ZERO key and waited for a reading from the instrument. The blank was removed and the above procedure was repeated for each of the samples.

4.7 Metals Analysis

4.7.1 Test Methods/Procedures

The interactions of metals/heavy metals within the soil matrix and aqueous samples, before and after biological treatment, were determined using various soil testing

techniques. A metals profile was generated which helped determine whether the soil/mud has met soil/groundwater quality standards by the La DEQ and the Italian government. The phosphorus soil test comprises of the following: (i) Extractant: 0.03 M NH_4F + 0.1 M HCl, (ii) Routine soil test: 2.5 g of soil to 50 mL solution, 15 min shaking, (iii) and analyzed on ICP (Inductively Coupled Plasma Atomic Absorption) (Page et al, 1982, p.403-427). Sodium, potassium, magnesium, and calcium soil tests are as follows: (i) Extractant: pH 7, 1 M NH_3OAc, (ii) Routine soil test: 2.5 g soil to 25 mL solution, 15 min shaking, and (iii) and analyzed on ICP (Page et al, 1982, p.159-164). The pH of the soil was determined by the following: (i) 35 g soil to 35 mL deionized water, 2 hr equilibration, and (ii) analyzed using a pH meter and electrode (Page et al, 1982, p.199-209). Manganese, iron, copper, and zinc soil tests are as follows: (i) Extractant: pH 7.3, 0.005 M DTPA (Diethylenetriaminepentaacetic acid), (ii) Routine soil test: 10 g soil to 20 mL solution, 2 hr. shaking, and (iii) analyzed by ICP (Page et al, 1982, p.323-334). Arsenic, cadmium, nickel, lead, and zinc soil tests are as follows: (i) Extractant: 0.1 M HCl, (ii) Routine soil test: 2.5 g soil to 25 mL solution, 15 min shaking, and (iii) Analyzed by ICP (Page et al, 1982, p. 323-334, 385-400).

The purpose behind a DTPA soil method (chelating agent) test was that it offered the most favorable combination of stability constants for the simultaneous complexing of Fe, Mn, Zn, and Cu. However, the stability constants for Ni and Cd like that for Zn are intermediate between those for Cu and Mn, making the DTPA equally suitable for these metals. The 0.1 N HCl method was developed in which Zn availability to acid-extractable Zn and "titratable alkalinity" values. In acid soils, 0.1 N HCl will extract Cu, Zn, Ni, and Cd held by organic matter, which chelates the metals as they are added either

as fertilizers, sprays such as Bordeaux mixture, or as components of municipal or industrial wastes (Page et al, 1982, p.331-333).

4.8 Nutrient Analysis

4.8.1 Ammonia Determination

4.8.1.1 Test Method

The Ammonia CHEMets® test method employs direct nesslerization. In a strongly alkaline solution, ammonia reacts with Nessler Reagent (K_2HgI_4) to produce a yellow-colored complex in direct proportion to the ammonia concentration. Results are expressed in ppm (mg/L) NH_3-N.

This method is applicable to drinking water, clean surface water, and good quality nitrified wastewater effluent. Other types of samples may require a preliminary distillation step. Ketones, alcohols, and aldehydes may cause off-color test results. Glycine and hydrazine will cause high test results. Aromatic and aliphatic amines, as well as iron, sulfide, calcium and magnesium, may cause turbidity and affect the test results.

4.8.1.2 Test Procedure

Recycled effluent from the microcosm was pipeted into a sampling cup, using dilutions accordingly, up to 25 ml mark. Two drops of A-1500 Stabilizer Solution was added to the sample, which was then stirred briefly with the tip of the ampoule to mix the contents of the sample cup. The CHEMet ampoule was placed in the sample cup, where the tip was broken off by applying pressure against the side of the cup. The ampoule was filled up leaving a small bubble to facilitate mixing. The contents in the ampoule were mixed by inverting it several times, allowing the bubble to travel from end to end each

time. The liquid from the exterior of the ampoule was wiped off and then let stand for 1 min. for color development. A high range comparator (1-10 mg/L) with various concentrations was used to determine the level of ammonia-nitrogen (NH_3-N) in the sample. If the color of the CHEMet ampoule is between two color standards, a concentration estimate can be made. The high range comparator was held nearly in a horizontal position while standing directly beneath a bright source of light. The CHEMet ampoule was placed between the color standards moving it from left to right along the comparator until the best color match was found. If the sample was highly concentrated (>10 mg/L), a new sample had to be collected and diluted accordingly to get within range of the comparator following the same procedures above.

4.8.2 Phosphate Determination

4.8.2.1 Test Method

The Phosphate CHEMets test method employs the stannous chloride chemistry. In an acidic solution, ortho-phosphate reacts with ammonium molybdate to form molybdophosphoric acid, which is then reduced by stannous chloride to the intensely colored molybdenum blue. The resulting blue color is directly proportional to the phosphate concentration. Results are expressed in ppm (mg/L) PO_4. Condensed phosphates (pyro-, meta-, and other polyphosphates) and organically bound phosphates do not respond to this test. Sulfide, thiosulfate, and thiocyanate will cause low test results.

4.8.2.2 Test Procedure

Recycled effluent from the microcosm was pipeted into a sampling cup, using dilutions accordingly, up to 25 ml mark. Two drops of A-8500 Activator Solution was

added to the sample, which was then stirred briefly with the tip of the ampoule to mix the contents of the sample cup. The CHEMet ampoule was placed in the sample cup, where the tip was broken off by applying pressure against the side of the cup. The ampoule was filled up leaving a small bubble to facilitate mixing. The contents in the ampoule were mixed by inverting it several times, allowing the bubble to travel from end to end each time. The liquid from the exterior of the ampoule was wiped off and then let stand for 2 min. for color development. A high range comparator (1-10 mg/L) with various concentrations was used to determine the level of ortho-phosphate in the sample. If the color of the CHEMet ampoule is between two color standards, a concentration estimate can be made. The high range comparator was held nearly in a horizontal position while standing directly beneath a bright source of light. The CHEMet ampoule was placed between the color standards moving it from left to right along the comparator until the best color match was found. If the sample was highly concentrated (>10 mg/L), a new sample had to be collected and diluted accordingly to get within range of the comparator following the same procedures above.

4.8.3 Nitrate Determination

4.8.3.1 Test Procedure

Recycled effluent from the microcosm was pipeted into a sampling cup, using dilutions accordingly, up to 15 ml mark. The contents of one A-6900 Cadmium Foil Pack was emptied into the sample cup. The sample cup was capped and shaken vigorously for exactly 3 min. Upon completion, the sample was allowed to sit undisturbed for 30 sec. The CHEMet ampoule was placed in the sample cup, where the tip was broken off by applying pressure against the side of the cup. The ampoule was

filled up leaving a small bubble to facilitate mixing. The contents in the ampoule were mixed by inverting it several times, allowing the bubble to travel from end to end each time. The liquid from the exterior of the ampoule was wiped off and then let stand for 10 min. for color development. A high range comparator (1-10 mg/L) with various concentrations was used to determine the level of nitrate-nitrogen (NO_3-N) in the sample. If the color of the CHEMet ampoule is between two color standards, a concentration estimate can be made. The high range comparator was held nearly in a horizontal position while standing directly beneath a bright source of light. The CHEMet ampoule was placed between the color standards moving it from left to right along the comparator until the best color match was found. If the sample was highly concentrated (>5 mg/L), a new sample had to be collected and diluted accordingly to get within range of the comparator following the same procedures above.

4.8.3.2 Interferences

Samples containing nitrite will give erroneous, high test results. Samples containing in excess of 2000 mg/L chloride will give low test results. Certain metals, chlorine, oil, and grease will also give low test results.

4.9 Residual Toxicity Analysis of Leachable Fractions

4.9.1 Principle of Procedure

Microtox® is used as an assessment tool to evaluate the relative toxicity of the recycled water collected from each microcosm (Phase two). Microtox® is a toxicity test that measures a decrease in light output of the luminescent bacterium, *Vibrio fischeri*. The presence of a toxicant causes a disruption in cellular respiration of the bacteria, and subsequently a decrease in light output. The decrease in light output was

measured by the toxicity analyzer (Microtox® Model 500). The results from the assay are given in effective concentration, or EC_{50} values. EC_{50} values are the effective concentration that causes a 50% decrease in light output in a 5 and 15 min. exposure period. A higher effective concentration correlates to a lower toxicity (Blackman, 2001).

4.9.2 Sample Procedure

Primary dilutions were first carried out to determine the amount of toxicant that could be measured effectively by the analyzer. Once the correct dilutions were determined, the procedure can be started. The machine was plugged in and clean, unused cuvettes were placed in each well. One thousand ul of diluent was pipetted into wells B1-B5, D1-D5, and F1-F5. One thousand ul of diluent was pipetted into wells A1-A4, C1-C4, and E1-E4. One thousand ul of reconstitution solution was pipetted into the reagent well. Two hundred fifty ul of osmotic adjusting solution was pipetted into wells A5, C5, and E5. Two and a half ml of the sample was added to A5 and mixed with the pipette. One ml was transferred from A5 to A4, 1 ml from A4 to A3, and 1 ml from A3 to A2 and A1 will serve as the blank. Two and a half ml of a second sample can be pipetted into well C5, and the remainder of the procedure carried out. A third sample should be placed in well E5. The Microtox reagent was taken out of the freezer and the reconstitution solution from the reagent well was added to it. The mixture was swirled and replaced into the cuvette with a pipette. Twenty ul of the reconstituted reagent was transferred to B1-B5, D1-D5, and F1-F5. A timer was set for 15 minutes (Blackman, 2001).

At the end of 15 minutes, B1 was first placed in the turret and the set button was pushed and then B1 was put in the turret and the read button was pushed. Cuvettes B2-

B5, as well as D1-D5 and F1-F5 are read in the same manner. Readings are recorded as I_o values. The timer was set for another 15 min., at which time transfers were begun. Five hundred ul is transferred from A1 to B1, A2 to B2, A3 to B3, A4 to B4, and A5 to B5. The same procedure was followed for C1-C5 into D1-D5 and for E1-E5 into F1-F5. The timer was set for 15 min. At 5 min., readings for B1-B5, D1-D5, and F1-F5 were taken and recorded as I_5. The cuvettes were immediately replaced. Readings were taken again at the end of 15 minutes and recorded as I_{15}. It is important to take readings in an expedient manner and in the correct order. This procedure was repeated for all samples (Blackman, 2001).

4.9.3 Calculations

Raw data was entered into Microtox® computer software, which automatically calculated the EC_{50} values for the recycled water samples.

5. RESULTS AND DISCUSSION

5.1 Phase One: Screening Study

5.1.1 Galliano Soil Samples

5.1.1.1 Total Petroleum Hydrocarbon Profiles: Phase One Study

Table 5.1 shows the total petroleum hydrocarbon concentration of control topsoil and drilling mud from Galliano, Sicily. Drilling mud and topsoil samples were randomly chosen in duplicate. Samples were obtained in triplicate for further research analyses, attempting to obtain a tighter statistical average due to the heterogeneity of the soil. This data was obtained using EPA Method 418.1 found in section 4.2. The concentration of the topsoil and drilling mud were 512.5±53.0 and 5000.0±530.0 mg/kg, respectively.

Table 5.2 shows reduction of total petroleum hydrocarbon concentration for the treated drilling mud. A 5 d bioreactor experiment was designed where the soil was mixed via a rotary blade. The execution of the experiment occurred once a drilling mud profile was obtained by GC-MS. Drilling mud samples were obtained in triplicate from the first container for assessment of remediation. Residual sample obtained at the end of the experiment was placed into 3 collection jars each with a volume of 250 ml.

Table 5.1: Total petroleum hydrocarbon profile of control topsoil and drilling mud: Phase one study*

Sample	*Absorbance*	*Dilution*	*Concentration (mg/kg)*
S I	0.172	2	550.0
S II	0.151	2	475.0
Average	----------	----------	512.5±53.0
DM I	0.329	10	5375.0
DM II	0.287	10	4625.0
Average	----------	----------	5000.0±530.0

*S=Control soil, DM=Drilling mud. (EPA Method 418.1, duplicate analyses of duplicate sets)

Table 5.2: Total petroleum hydrocarbon profile of biotreated drilling mud: Phase one study*

Sample	*Absorbance*	*Dilution*	*Concentration (mg/kg)*	*Efficiency (%)*
Jar I i	0.295	1	450.0	91.0
Jar I ii	0.210	2	600.0	88.0
Jar I iii	0.272	2	800.0	84.0
Average	-------	-------	617.0±176.0	87.7±3.5
Jar II i	0.184	1	238.0	95.2
Jar II ii	0.326	1	475.0	90.5
Jar II iii	0.077	10	625.0	87.5
Average	-------	-------	446.0±195.0	91.1±3.9
Jar III i	0.225	2	675.0	86.5
Jar III ii	0.349	1	525.0	89.5
Jar III iii	0.273	1	400.0	92.0
Average	------	-------	533.0±138.0	89.3±2.8

*(EPA Method 418.1, triplicate analyses of triplicate sets)

Approximately 804 g of sediment was preserved. Samples were analyzed using EPA Method 418.1. In Jars I-III, the biotreated mud reduced to 617.0±176.0, 446.0±195.0, and 533.0±138.0 from 5000.0±530.0 mg/kg, respectively. Efficiency of biodegradation for the drilling mud resulted in a reduction of 84.2-95% total petroleum hydrocarbons. Aqueous and solid samples were preserved for metals analysis. Figure 5.1 shows the overall trend of the total petroleum hydrocarbon content between control and experimental samples from Galliano, Sicily.

Table 5.3 shows a total petroleum hydrocarbon profile for control and experimental drilling mud using GC-FID. Data was calculated according to the formula found in Section 4.4.4. Control drilling mud samples were diluted 1:10 before injection through the column. Concentration of the drilling mud within containers I, II, and III were 576.0±286.1, 89.1±71.4, and 46.4±15.3 mg/kg, respectively. Concentration

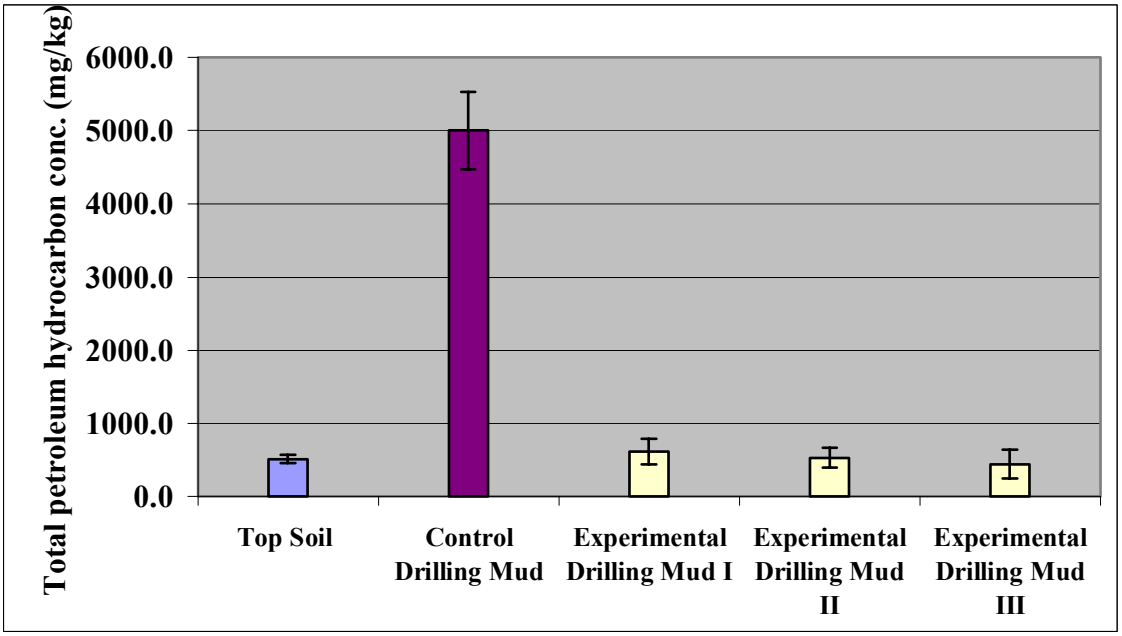

Figure 5.1: Total petroleum hydrocarbon concentrations of topsoil and drilling mud after 5 days treatment: Phase one study. (EPA Method 418.1, triplicate analyses of triplicate sets)

Table 5.3: Total petroleum hydrocarbon profile of control and experimental drilling mud: Phase one study*

Sample	*(mg/kg)*
Control DM I	576.0±286.1
Control DM II	89.1±71.4
Control DM III	46.4±15.3
Topsoil I	8.9±5.3
Topsoil II	4.8±3.4
Topsoil III	5.8±3.1
Treated DM	24.0±7.0
	(mg/L)
Aqueous Phase	0.75±0.11

*DM=Drilling mud, I, II, and III= Container number (EPA Method SW-846 8015B, triplicate analyses of triplicate sets)

of the topsoil within containers I, II, and III were 8.9±5.3, 4.8±3.4, and 5.8±3.1 mg/kg, respectively. The treated drilling mud had a net concentration of 24.0±7.0 mg/kg. The extract obtained at the end of the experiment had a net concentration of 0.75±0.11 mg/L. The drilling mud concentration went from 576.0±286.1 to 24.0±7.0 mg/kg over the 5d period, which resulted in a biodegradation efficiency of 95.8%. Less than 1 mg/L of petroleum hydrocarbons were present in the aqueous phase.

5.1.1.2 Polycyclic Aromatic Hydrocarbons Residuals: Phase One Study

Various concentrations of PAH and phenols were analyzed for the control and experimental samples from Galliano, Sicily. The samples were diluted accordingly and an internal standard was added to each sample to determine the components of the drilling mud. The polycyclic aromatic hydrocarbon tables generated show a breakdown of the specific compounds present in the samples analyzed. Table 5.4 shows the polycyclic aromatic hydrocarbon profile of control drilling mud. Net concentration of the control drilling mud in containers I, II, and III were 820.2±355.2, 175.2±16.2, and 162.7±46.9 ug/kg, respectively. The various components present in the control drilling mud were phenol, 2,4-dichlorophenol (Container I), naphthalene, 2-methylnaphthalene, acenaphthene, dibenzofuran, fluorene, phenanthrene, anthracene, carbazole (Container I), and fluoranthene (Container I). Table 5.5 shows the polycyclic aromatic hydrocarbon profile for the topsoil. The topsoil had a net concentration of 8.2±1.7, 7.6±1.8, and 8.5±2.8 ug/kg, respectively. The topsoil PAH and phenol concentration was 10-100 times lower than the drilling mud. Table 5.6 shows the polycyclic aromatic hydrocarbon profile for the treated drilling mud. The treated drilling mud had a net concentration of 4.15±0.54 ug/kg. After biological treatment, a 39.3% reduction in phenol occurred, 2,4-

Table 5.4: Polycyclic aromatic hydrocarbon profile of control drilling mud: Phase one study*

Control Drilling mud	*DM I*	*DM I*	*DM II*	*DM II*	*DM III*	*DM III*
Compound	*(i)*	*(ii)*	*(i)*	*(ii)*	*(i)*	*(ii)*
Phenol	2.65	1.57	0.00	3.16	1.95	16.85
2-chlorophenol	0.00	0.00	0.00	0.00	0.00	0.00
2-Methylphenol (o-cresol)	0.00	0.00	0.00	0.00	0.00	0.00
2-Methylphenol (p-cresol)	0.00	0.00	0.00	0.00	0.00	0.00
2,4-Dimethylphenol	0.00	0.00	0.00	0.00	0.00	0.00
2,4-Dichlorophenol	20.36	10.23	0.00	0.00	0.00	0.00
Naphthalene	17.69	15.86	9.86	17.17	21.76	13.48
4-Cl-3-methylphenol	0.00	0.00	0.00	0.00	0.00	0.00
2-Methylnaphthalene	273.93	152.01	50.76	49.47	80.83	41.37
2,4,6-Trichlorophenol	0.00	0.00	0.00	0.00	0.00	0.00
Acenaphthylene	0.00	0.00	0.00	0.00	0.00	0.00
Acenaphthene	38.16	20.92	11.18	8.89	17.47	8.18
Dibenzofuran	42.46	21.75	10.86	8.60	11.91	6.36
2,3,4,6-Tetrachlorophenol	0.00	0.00	0.00	0.00	0.00	0.00
Fluorene	100.35	51.16	13.96	10.59	11.62	5.60
Pentachlorophenol	0.00	0.00	0.00	0.00	0.00	0.00
2,4-Dinitrophenol	0.00	0.00	0.00	0.00	0.00	0.00
Phenanthrene	271.09	136.73	42.83	31.99	24.45	17.95
Anthracene	284.03	149.99	47.24	33.86	25.89	19.82
Carbazole	9.07	0.00	0.00	0.00	0.00	0.00
Fluoranthene	11.60	8.82	0.00	0.00	0.00	0.00
Benzo (a) Anthracene	0.00	0.00	0.00	0.00	0.00	0.00
Chrysene	0.00	0.00	0.00	0.00	0.00	0.00
Benzo (b) fluoranthene	0.00	0.00	0.00	0.00	0.00	0.00
Benzo (k) fluoranthene	0.00	0.00	0.00	0.00	0.00	0.00
Benzo (a) pyrene	0.00	0.00	0.00	0.00	0.00	0.00
Dibenzo (a,h) anthracene	0.00	0.00	0.00	0.00	0.00	0.00
Indeno (1,2,3-cd) pyrene	0.00	0.00	0.00	0.00	0.00	0.00
Total PAH (ug/kg)	23.0	11.79	0	3.16	1.95	16.85
Total Phenol (ug/kg)	1048.37	557.23	186.69	160.56	193.92	112.75
Total PAH & Phenol (ug/kg)	1071.38	569.03	186.69	163.71	195.87	129.60

*DM=Drilling mud, I, II, and III=Container number, PAH=Polycyclic aromatic hydrocarbons. (EPA Method SW-846 8270C, duplicate analyses of duplicate sets)

Table 5.5: Polycyclic aromatic hydrocarbon profile of control topsoil: Phase one study*

Control Topsoil	*S I*	*S I*	*S I*	*S II*	*S II*	*S II*	*S III*	*S III*	*S III*
Compound	*(i)*	*(ii)*	*(iii)*	*(i)*	*(ii)*	*(iii)*	*(i)*	*(ii)*	*(iii)*
Phenol	1.73	2.22	1.51	1.20	6.15	1.06	1.66	1.29	0.69
2-chlorophenol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-Methylphenol (o-cresol)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-Methylphenol (p-cresol)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2,4-Dimethylphenol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2,4-Dichlorophenol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Naphthalene	0.71	1.04	0.51	0.30	0.28	0.37	0.12	0.40	0.38
4-Cl-3-methylphenol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-Methylnaphthalene	1.96	2.32	1.24	0.63	0.60	0.95	0.00	0.95	0.82
2,4,6-Trichlorophenol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Acenaphthylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Acenaphthene	0.40	0.43	0.39	0.39	0.00	0.00	2.34	2.39	2.32
Dibenzofuran	0.53	0.59	0.46	0.43	0.25	0.48	0.00	0.33	0.27
2,3,4,6-Tetrachlorophenol	0.52	1.56	1.33	0.60	1.12	2.97	0.42	2.68	1.72
Fluorene	0.00	0.00	0.00	0.00	0.00	0.43	0.00	0.00	0.00
Pentachlorophenol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2,4-Dinitrophenol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Phenanthrene	1.75	1.87	1.43	2.17	0.83	1.65	0.35	1.30	1.60
Anthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.40	1.32	1.79
Carbazole	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fluoranthene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Benzo (a) Anthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chrysene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Benzo (b) fluoranthene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Benzo (k) fluoranthene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Benzo (a) pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dibenzo (a,h) anthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Indeno (1,2,3-cd) pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total PAH (ug/kg)	5.87	7.80	5.35	4.52	3.07	6.86	3.63	9.39	8.90
Total Phenol (ug/kg)	1.73	2.22	1.51	1.20	6.15	1.06	1.66	1.29	0.69
Total PAH & Phenol (ug/kg)	7.60	10.02	6.86	5.72	9.22	7.92	5.29	10.68	9.59

*S=Topsoil, I, II, and III=Container number, PAH=Polycyclic aromatic hydrocarbons. (EPA Method SW-846 8270C, triplicate analyses of triplicate sets)

Table 5.6: Polycyclic aromatic hydrocarbon profile of biotreated drilling mud: Phase one study*

Biotreated Drilling Mud	*Bio DM I*	*Bio DM II*	*Bio DM III*
Compound			
Phenol	1.43	1.13	0.99
2-chlorophenol	0.11	0.00	0.00
2-Methylphenol (o-cresol)	0.00	0.00	0.00
2-Methylphenol (p-cresol)	0.00	0.00	0.00
2,4-Dimethylphenol	0.00	0.00	0.00
2,4-Dichlorophenol	0.00	0.00	0.00
Naphthalene	0.36	0.28	0.35
4-Cl-3-methylphenol	0.00	0.00	0.00
2-Methylnaphthalene	0.91	0.68	0.84
2,4,6-Trichlorophenol	0.00	0.00	0.00
Acenaphthylene	0.00	0.00	0.00
Acenaphthene	0.00	0.00	0.00
Dibenzofuran	0.26	0.19	0.22
2,3,4,6-Tetrachlorophenol	1.10	0.79	0.97
Fluorene	0.00	0.00	0.00
Pentachlorophenol	0.00	0.00	0.00
2,4-Dinitrophenol	0.00	0.00	0.00
Phenanthrene	0.57	0.58	0.70
Anthracene	0.00	0.00	0.00
Carbazole	0.00	0.00	0.00
Fluoranthene	0.00	0.00	0.00
Benzo (a) Anthracene	0.00	0.00	0.00
Chrysene	0.00	0.00	0.00
Benzo (b) fluoranthene	0.00	0.00	0.00
Benzo (k) fluoranthene	0.00	0.00	0.00
Benzo (a) pyrene	0.00	0.00	0.00
Dibenzo (a,h) anthracene	0.00	0.00	0.00
Indeno (1,2,3-cd) pyrene	0.00	0.00	0.00
Total PAH (ug/kg)	2.09	1.73	2.11
Total Phenol (ug/kg)	2.64	1.92	1.97
Total PAH & Phenol (ug/kg)	4.73	3.65	4.08

*Bio DM=Treated drilling mud, PAH=Polycyclic aromatic hydrocarbons.
(EPA Method SW-846 8270C, triplicate analyses of triplicate sets)

dichlorophenol completely degraded, a 98.0% reduction in naphthalene occurred, a 99.6% reduction in 2-methylnaphthalene occurred, acenaphthene completely degraded, a 99.3% reduction in dibenzofuran occurred, 0.95±0.16 ug/kg of 2,3,4,6-tetrachlorophenol was present in the biotreated drilling mud, fluorene completely degraded, a 99.7% reduction in phenanthrene occurred, and anthracene, carbazole and fluoranthene all completely degraded. The experiment showed a 97-99.5% reduction in PAH and phenols within the treated drilling mud.

5.1.1.3 Residual Metals Analysis: Phase One Study

Table 5.7 shows the Italian and La DEQ soil standards for particular metal compounds and various hydrocarbons. Table 5.8 shows the metals profile for the topsoil, control and experimental drilling mud from Galliano, Sicily. The samples analyzed were performed in duplicate. A significant change in Na occurred in the experimental drilling mud, which went from 6495.0±366.3 to 437.5±54.4 mg/kg. More abundant levels of Fe were present in the experimental drilling mud, which went from 86.0±4.9 to 170.3±1.7 mg/kg over the 5d period. In both Zn analyses, a chelating agent DTPA and HCl were used as part of standard methods. The concentration of Zn for the drilling mud had lowered from 78.9±4.2 and 59.2±0.7 mg/kg to 47.6±3.4 and 32.4±0.7 mg/kg, respectively. The concentration of Zn for the topsoil was 0.84±0.45 mg/kg, significantly different from the drilling mud. The Pb levels for the drilling mud lowered from 1.3±0.1 mg/kg to 0.49±0.01 mg/kg over the 5d period. The aqueous phase was analyzed and the data indicates that metals have a high affinity to the soil particles rather than the aqueous phase (Page et al, 1982).

Table 5.7: Italian and La DEQ soil standards

Metals	*Italian Residential/ Agricultural Limits (mg/kg)*	*Italian Industrial Limits (mg/kg)*	*La DEQ Non-Industrial Limits* (mg/kg)*	*La DEQ Industrial Limits* (mg/kg)*
Sb	10	30	30	750
As	20	50	0.38	3
Be	2	10	150	3700
Cd	2	15	37	940
Co	20	250	4500	110000
Cr^{+3}	150	800	110000	1000000
Cr^{+6}	2	15	220	5600
Hg	1	5	22	560
Ni	120	500	1500	37000
Pb	100	1000	400	1700
Se	3	15	370	9400
V	90	250	520	13000
Zn	150	1500	22000	560000
F	100	2000	1800	31000
Benzene	0.1	2	1.5	3.2
Ethyl Benzene	0.5	50	1500	13000
Styrene	0.5	50	4500	41000
Toluene	0.5	50	690	4800
Xylene	0.5	50	12000	83000
Benzo (a) pyrene	0.1	10	0.33	0.36
Chrysene	5	50	61	400
Pyrene	5	50	1500	27000
Benzo (a) Anthracene	0.5	10	0.56	3.6
Benzo (b) Fluoranthene	0.5	10	0.56	3.6
Benzo (k) Fluoranthene	0.5	10	5.5	35
Dibenzo (a) anthracene	0.1	10	0.33	0.36

*Reference: La DEQ RECAP 2000 Table 2 Management Option I Standards for Soil.
La DEQ=Louisiana Department of Environmental Quality
RECAP=Risk Evaluation/Corrective Action Program

Table 5.8: Metals profile of topsoil, control and experimental drilling mud, and aqueous phase: Phase one study*

Metals	*Top soil (mg/kg)*	*Control drilling mud (mg/kg)*	*Experimental drilling mud (mg/kg)*	*Aqueous Phase (mg/L)*
P	33.0±8.5	37.0±5.7	160.5±17.7	0.32±0.01
Na	554.0±11.3	6495.0±366.3	437.5±54.4	350.9±1.3
K	272.0±15.6	324.5±13.4	420.5±67.2	----------
Ca	6703.0±239.0	4238.0±479.4	4836.5±652.7	188.3±3.0
Mg	478.0±12.7	364.0±5.7	299.0±45.3	26.4±0.1
Cu	2.5±0.1	3.6±0.1	3.5±0.3	0.07±0.01
Fe	37.7±8.2	86.0±4.9	170.3±1.7	2.56±0.01
Mn	8.7±4.2	36.6±1.0	44.5±0.6	1.15±0.01
Zn**	8.5±0.1	78.9±4.2	47.6±3.4	0.02±0.01
As	DL	DL	DL	----------
Cd	0.03±0.01	0.17±0.01	0.05	0.01±0.01
Ni	0.32±0.05	1.33±0.02	1.36±0.02	0.02±0.01
Pb	0.10	1.3±0.1	0.49±0.01	0.04
Zn***	0.84±0.45	59.2±0.7	32.4±0.7	----------
Cr	----------	----------	----------	0.01±0.01
Al	----------	----------	----------	0
Si	----------	----------	----------	5.61±0.05

*DL=Detection Limit (0.06 mg/kg, 2 g soil in 20 ml hydrochloric acid)
Reference: (Page et al, 1982) Shaded value denotes significant change (P<0.05).
**DTPA (Diethylenetriaminepentaacetic acid) Method. ***HCl Method. (Triplicate analyses of triplicate sets)

5.1.1.4 Soil Microbial Data: Phase One Study

Microbial data was obtained for experimental drilling mud from Galliano, Sicily. Abundant microbial colonies were noted which favored the breakdown of PAH's and phenols in the organic fraction of the soil. A higher dilution factor was necessary to obtain the appropriate enumeration of the microbes. Two experimental samples were analyzed. The average count for experimental samples was $2.66e^{+6}$±$1.9e^{+5}$ and $2.37e^{+6}$±$4.5e^{+5}$ colony forming units per ml (CFU/ml). Experimental samples had a dilution factor of 10^{-4}. Samples were prepared and plated found in Section 4.3.

5.1.1.5 Leachate Residual Analysis: Phase One Study

Table 5.9 shows the chemical oxygen demand of control and experimental extracts from the 14d leachability test. Chemical oxygen demand for the control sample went from 3.0±4.2 mg/L to 24.0±5.6 mg/L. Chemical oxygen demand for the experimental sample went from 5.0±5.6 mg/L to 21.0±15.5 mg/L and then decreased to 12.0±0.0 mg/L. The values obtained for both control and experimental samples indicated that an increase in chemical oxygen demand was not detectable until after one week of saturation. Both control and experimental samples had a COD measurement <30 mg/L. Figure 5.2 show the chemical oxygen demand trend for control versus experimental drilling mud treatments with standard deviations.

Table 5.10 shows the Italian and La DEQ leachate residual standards for groundwater. The table is a key to what are acceptable leachate limits of particular metals for any environmental system. Table 5.11 shows the residual metal concentrations obtained for the untreated sample (effluent) upon completion of the Italian leachability

Table 5.9: Chemical oxygen demand (COD) of control and experimental aqueous phase aliquots: Phase one study*

Time (hrs.)	*Control sample (mg/L)*	*Experimental sample (mg/L)*
2	3.0±4.2	5.0±5.6
8	3.0±2.8	0.0
24	3.0±1.4	2.0±1.4
48	6.5±0.7	0.5±0.7
72	7.0±2.8	4.0±4.2
102	4.5±0.7	2.5±2.1
164	11.0±1.4	21.0±15.5
336	24.0±5.6	12.0±0.0

*(Method 8000 for water, wastewater and seawater, duplicate analyses of duplicate sets)

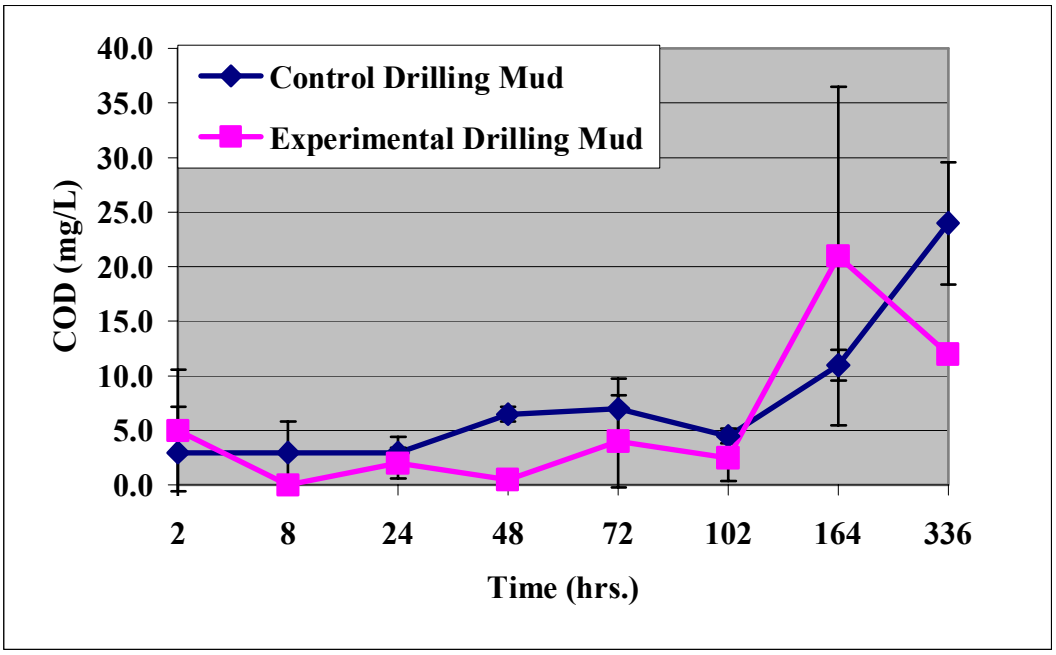

Figure 5.2: Variations in chemical oxygen demand (COD): Control vs. Experimental drilling mud treatments-Phase one study (Method 8000 for water, wastewater and seawater, duplicate analyses of duplicate sets)

test. Cadmium went from 0.047 mg/L to 0.003 mg/L and selenium stayed consistent at 0.031 mg/L. Cadmium had exceeded both Italian and La DEQ limits measured at 0.005 mg/L. Selenium exceeded Italian limits measured at 0.010 mg/L, but did not exceed La DEQ limits at 0.050 mg/L. All metals had a concentration <1 mg/L over all time periods except iron, which had 1.19 mg/L upon completion.

Table 5.12 shows the residual metal concentrations for the biotreated sample (effluent). Cadmium went from 0.029 mg/L to 0.003 and gradually rose to 0.076 mg/L and selenium stayed consistent at 0.031 mg/L. Cadmium exceeded Italian and La DEQ limits measured at 0.005 mg/L at portions of the experiment. Upper limits of detection for As, Ba, Cd, Cr, Pb, and Se were 1.15 mg/L, Hg at 0.0058 mg/L, Ag at 1.2 mg/L, and Fe at 1.27 mg/L. Lower limits for As, Ba, Cd, Cr, Fe, Pb, and Se were 0.85 mg/L, Hg at 0.0043 mg/L, and Ag at 0.3 mg/L (Analytical and Environmental Testing, Inc., 2001).

Table 5.10: Italian and La DEQ groundwater standards*

Unit of Measurement	*Italian Concentration Limit*	*La DEQ Groundwater Standards**
NO_3 (mg/L)	50	10
F (mg/L)	1.5	0.24
SO_4 (mg/L)	250	-------
Cl (mg/L)	200	-------
Ba (mg/L)	1	2
Zn (mg/L)	3	11
Be (µg/L)	10	4
Co (µg/L)	250	2200
Ni (µg/L)	10	730
V (µg/L)	250	260
As (µg/L)	50	50
Cd (µg/L)	5	5
Cr^{+3} (µg/L)	50	55000
Pb (µg/L)	50	15
Se (µg/L)	10	50
Hg (µg/L)	1	2
COD (mg/L)	30	<30
pH	5.5<>12.0	-------

*Reference: La DEQ RECAP 2000 Table 3 Management Option I Standards for Groundwater.

Table 5.11: Metals speciation of leachable fraction: Control drilling mud Phase one study*

Time	2 h	8 h	24 h	48 h	72 h	102 h	164 h	336 h	Italian Standard	La DEQ Standard
Metal	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
As	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.050	0.050
Ba	0.042	0.035	0.099	0.163	0.400	0.543	0.561	0.636	1.000	2.000
Cd	0.047	0.023	0.017	0.026	0.025	0.012	0.007	0.003	0.005	0.005
Cr	0.039	0.029	0.031	0.043	0.041	0.021	0.005	0.025	0.050	0.110
Fe	0.873	0.377	0.510	0.038	0.206	0.449	0.445	1.19	----	----
Pb	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.050	0.015
Hg	0.0002	0.0002	0.0002	0.0003	0.0002	0.0002	0.0002	0.0002	0.0010	0.0020
Se	0.031	0.031	0.031	0.031	0.031	0.031	0.031	0.031	0.010	0.050
Ag	0.003	0.013	0.004	0.016	0.011	0.003	0.003	0.003	----	----

*White denotes less than (<), shaded denotes excedence ($P<0.05$). (Methods 6010A and 7470A, U.S. EPA, 1983).

Table 5.12: Metals speciation of leachable fraction: Biotreated drilling mud Phase one study*

Time	2 h	8 h	24 h	48 h	72 h	102 h	164 h	336 h	Italian Standard	La DEQ Standard
Metal	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
As	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.050	0.050
Ba	0.026	0.029	0.037	0.050	0.077	0.110	0.123	0.115	1.000	2.000
Cd	0.029	0.012	0.018	0.003	0.003	0.007	0.048	0.076	0.005	0.005
Cr	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.050	0.110
Fe	0.041	0.005	0.005	0.005	0.178	0.021	0.005	0.158	----	----
Pb	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.050	0.015
Hg	0.0003	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0010	0.0020
Se	0.031	0.031	0.031	0.031	0.031	0.031	0.031	0.031	0.010	0.050
Ag	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	----	----

*White denotes less than (<), shaded denotes excedence (P<0.05). (Methods 6010A and 7470A, U.S. EPA, 1983).

5.1.2 Syracuse Soil Samples

5.1.2.1 Total Petroleum Hydrocarbon Profiles: Phase One Study

Table 5.13 shows the total petroleum hydrocarbon content of control and experimental samples from Syracuse, Sicily. Total petroleum hydrocarbon content reduced from 2375.0±696.0 to 1291.7± 314.6 mg/kg after biological treatment, resulting in a biodegradation efficiency of 54.4%. Figure 5.3 shows reduction of total petroleum hydrocarbon concentration in the Syracuse sample graphically.

5.1.2.2 Polycyclic Aromatic Hydrocarbons Residuals: Phase One Study

Various concentrations of PAH and phenols were analyzed for the control sample from Syracuse, Sicily. The polycyclic aromatic hydrocarbon table generated represents a chemical profile of the specific compounds present. Table 5.14 shows the polycyclic aromatic hydrocarbon profile for control and experimental Syracuse soil samples. The results indicate that the concentration of the soil went from 6830.4±1064.9 to 1687.2±9.9

Table 5.13: Total petroleum hydrocarbon profile of control and experimental Syracuse samples: Phase one study*

Sample	*Absorbance*	*Dilution*	*Concentration (mg/kg)*
PF I	0.120	10	1625.0
PF II	0.175	10	2500.0
PF III	0.210	10	3000.0
Average	----------	----------	2375.0±696.0
Bio PF I	0.059	10	1000.0
Bio PF II	0.076	10	1250.0
Bio PF III	0.095	10	1625.0
Average	----------	----------	1291.7± 314.6

*Sample labeled "PF=Porto Franco." Biodegradation efficiency was 54.4%. (EPA Method 418.1, triplicate analyses of triplicate sets)

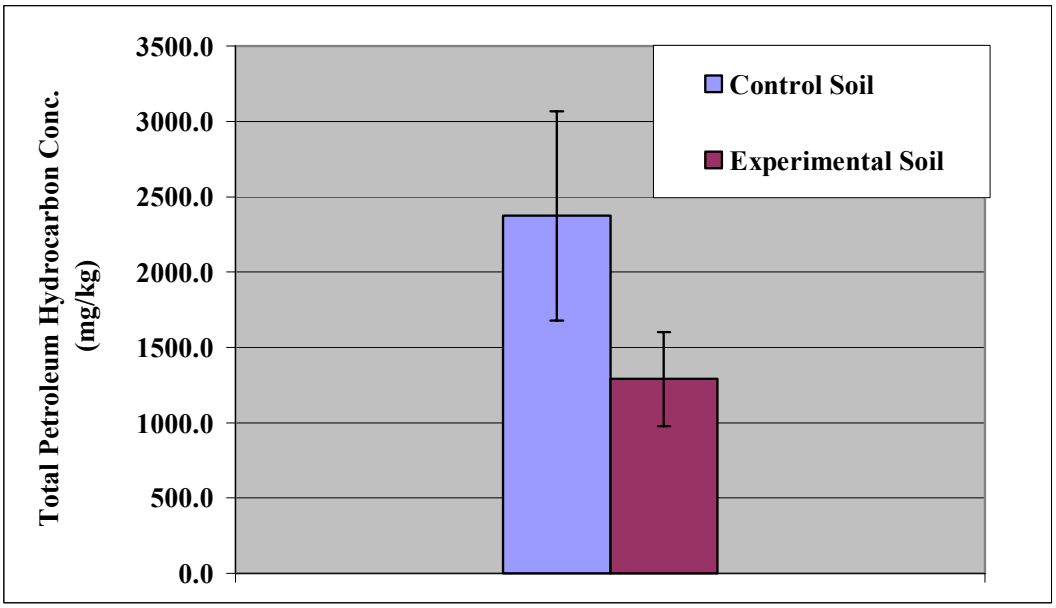

Figure 5.3: Total petroleum hydrocarbon reduction of Syracuse sample: Phase one study (EPA Method 418.1, triplicate analyses of triplicate sets)

ug/kg after biodegradation via mixing. The components of the control sample were phenol, naphthalene, 2-methylnaphthalene, acenaphthene, dibenzofuran, flourene, and flouranthrene. After biological treatment, naphthalene completely degraded, a 71.7% reduction in 2-methylnaphthalene occurred, acenaphthene completely degraded, a 65.9% reduction in dibenzofuran occurred, a 48.2% reduction in flourene occurred, and a 46.7% reduction in flouranthrene occurred. The experiment showed a 75.3% reduction in PAH and phenols within the treated soil.

5.1.2.3 Soil Microbial Data: Phase One Study

Microbial data was obtained for the control sample from Syracuse, Sicily (Section 4.3). Microbial counts were assessed to ascertain the amount of microbial activity in the soil before treatment. Average count for the control sample was 25600±3300 colony forming units per ml (CFU/ml). The control sample had a dilution factor of 10^{-2}.

5.1.3 General Discussion of Phase One Data

The results indicated a very favorable response in terms of a decline of TPH concentrations and an increase in microbial population for the site soils. The differentiation between the total petroleum hydrocarbon concentrations obtained from the spectrophotometer versus the GC-FID was due to the different calculations used in the protocols. Twenty-five ml of sample are utilized in the calculation for the spectrophotometer and 2 ul of sample was injected into the GC-FID. The PAH and phenol data for the treated drilling mud indicated that a reduction in straight chain hydrocarbons and alkanes occurred. The idea of bioremediation is to turn a toxic substance/compound to become more water-soluble because of its environmental persistence.

Table 5.14: Polycyclic aromatic hydrocarbon profile of control and experimental Syracuse samples: Phase one study*

Syracuse Samples: Before and After Treatment	*SBT*	*SBT*	*SBT*	*SAT*	*SAT*
Compound	*(i)*	*(ii)*	*(iii)*	*(i)*	*(ii)*
Phenol	25.58	27.90	34.29	0.00	0.00
2-chlorophenol	0.00	0.00	0.00	0.00	0.00
2-Methylphenol(o-cresol)	0.00	0.00	0.00	0.00	0.00
2-Methylphenol(p-cresol)	0.00	0.00	0.00	0.00	0.00
2,4-Dimethylphenol	0.00	0.00	0.00	0.00	0.00
2,4-Dichlorophenol	0.00	0.00	0.00	0.00	0.00
Naphthalene	929.16	722.88	960.31	0.00	0.00
4-Cl-3-methylphenol	0.00	0.00	0.00	0.00	0.00
2-Methylnaphthalene	6383.65	4581.75	5872.94	1593.00	1586.68
2,4,6-Trichlorophenol	0.00	0.00	0.00	0.00	0.00
Acenaphthylene	0.00	0.00	0.00	0.00	0.00
Acenaphthene	92.36	107.50	134.36	0.00	0.00
Dibenzofuran	50.97	52.04	65.63	17.23	21.10
2,3,4,6-Tetrachlorophenol	0.00	0.00	0.00	0.00	0.00
Fluorene	126.17	103.82	121.74	60.88	60.59
Pentachlorophenol	0.00	0.00	0.00	0.00	0.00
2,4-Dinitrophenol	0.00	0.00	0.00	0.00	0.00
Phenanthrene	0.00	0.00	0.00	0.00	0.00
Anthracene	0.00	0.00	0.00	0.00	0.00
Carbazole	0.00	0.00	0.00	0.00	0.00
Fluoranthene	39.01	29.94	29.18	23.08	11.82
Benzo(a)Anthracene	0.00	0.00	0.00	0.00	0.00
Chrysene	0.00	0.00	0.00	0.00	0.00
Benzo(b)fluoranthene	0.00	0.00	0.00	0.00	0.00
Benzo(k)fluoranthene	0.00	0.00	0.00	0.00	0.00
Benzo(a)pyrene	0.00	0.00	0.00	0.00	0.00
Dibenzo(a,h)anthracene	0.00	0.00	0.00	0.00	0.00
Indeno(1,2,3-cd)pyrene	0.00	0.00	0.00	0.00	0.00
Total PAH (ug/kg)	7,621.30	5597.90	7184.20	1694.20	1680.20
Total Phenol (ug/kg)	25.6	27.9	34.3	-	-
Total PAH & Phenol (ug/kg)	7,646.89	5625.84	7218.45	1694.18	1680.18

*SBT=Syracuse sample before treatment, SAT=Syracuse sample after treatment. (EPA Method SW-846 8270C, triplicate analyses of triplicate sets for control sample, duplicate analyses for treated sample)

The addition of microbes into a biological system or reactor vessel require an abundant nutrient source consisting of ammonium nitrate and potassium phosphate monobasic to help control the pH. With the addition of nutrients, this helped facilitate the microbes in degrading the organic fraction present in the soil. With enhanced microbial activity and the use of a rotary blade to homogenize the soil, the kinetic rate of degradation was accelerated and resulted in a significant decrease in heavy metal complexes. The Italian limits established for soil standards have tighter regulations compared to the United States for both industrial and non-industrial sites. Tighter regulations are necessary for residential and agricultural sites to help protect from any of the major routes of exposure such as inhalation, ingestion, or dermal contact (La DEQ RECAP 2000, Soil Standards).

Chemical oxygen demand (COD) was measured (i) To determine the extent of the leachable fraction of the soil into the aqueous phase and (ii) To assure that adequate U.S. and Italian water quality standards were met. According to U.S. and Italian government standards, a chemical oxygen demand 30 mg/L or less is acceptable for leachate standards. The data contains residual metal concentrations that have to be met and if the levels are not sufficient, remedial action has to be enforced. Management Option I was chosen for a reference point because it is the most cost-effective method and represents the minimal requirements necessary for adequate groundwater levels. If Management Option II or III were chosen through the La DEQ, it would require funds and stringent permitting to remediate the contaminants and heavy metals present in the water (La DEQ RECAP 2000, Groundwater Standards).

The results indicate there are two metals of concern for the Italians, cadmium and selenium, and for La DEQ standards, only cadmium, for both untreated and treated samples. The results also indicate that the biodegradation efficiency for the Syracuse sample was much lower due to its composition (sandy, coarse texture). The Galliano drilling mud is a highly viscous material containing clay particles. The Syracuse sample has very fine particle size thus water flows faster through it than through clay particles. Further research is needed for the Syracuse sample.

5.2 Phase Two: Microcosm Study

5.2.1 Total Petroleum Hydrocarbon Profiles: Phase Two Study

Table 5.15 shows the total petroleum hydrocarbon profile of the topsoil and drilling mud over a 40d period. Composite samples were obtained in triplicate during the course of the experiment. The GC-FID was used to monitor TPH trends for this study. The topsoil for control and experimental setups had an initial concentration <12.5 mg/kg and upon completion of the experiment, had lowered to <3.5 mg/kg. Approximately 70% reduction in total petroleum hydrocarbons occurred in the topsoil over the 40d period. Control I and II drilling mud went from 217.12±43.38 and 149.68±45.51 mg/kg to 15.16±3.35 and 34.27±15.86 mg/kg, respectively. Experimental I, II, and III drilling mud went from 89.20±67.42, 141.71±64.80, and 197.87±77.38 mg/kg to 5.24±6.15, 15.02±10.20, and 9.65±9.37 mg/kg, respectively. Toxicity assessment was performed on the sand layer to determine if a leachable fraction of the drilling mud into the sand layer was present. Less than 1.5 mg/kg of total petroleum hydrocarbons were present in the sand layer.

Figures 5.4 and 5.5 shows the drilling mud and topsoil trend for both control and experimental setups. Figure 5.6 shows a logarithmic scale of the reduction present in the drilling mud. The data shows almost one order of magnitude in reduction for both controls and two orders of magnitude reduction for experimental I. Experimental II and III had one order of magnitude reduction in total petroleum hydrocarbons. The efficiency of degradation for control and experimental test beds were 85.1±11.2% and 92.9±3.0%, respectively. The total weight of topsoil and drilling mud used in the experiment was approximately 25 kg/setup. By volume, 34L of drilling mud was utilized for treatment.

A statistical analysis (correlation) was determined for the drilling mud total petroleum hydrocarbon trend over time (Figure 5.4). Controls I and II had R-squared values of 0.6434 and 0.4737, respectively, which indicated that the sampling points obtained during the experiment were widely dispersed, showing large standard deviations in soil concentrations. Experimental microcosms I through III had R-squared values of 0.9751, 0.744, and 0.8913, respectively, which indicated that the sampling points obtained had a tighter statistical average with respect to soil concentration. Total petroleum hydrocarbon chromatograms were generated for control and experimental test beds at t=0, 40d to display the broad range of compounds present in the drilling mud (Appendices A and B).

Table 5.15: Total petroleum hydrocarbon profile of topsoil and drilling mud: Microcosm study*

Time	0d	7d	14d	28d	40d
Microcosm ID	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
Control I					
Top soil	6.25±1.90	1.86±0.34	2.49±2.47	1.28±0.55	0.86±0.70
Drilling mud	217.12±43.38	51.10±9.91	99.84±78.23	59.89±15.71	15.16±3.35
Control II					
Top soil	12.13±6.43	1.15±0.82	3.54±3.10	7.60±9.41	1.86±2.09
Drilling mud	149.68±45.51	55.11±34.56	138.00±61.31	52.19±24.36	34.27±15.86
Experimental I					
Top soil	4.03±1.96	1.52±0.87	2.14±0.20	2.71±1.11	1.42±0.82
Drilling mud	89.20±67.42	64.90±40.23	57.75±19.66	29.14±18.11	5.24±6.15
Experimental II					
Top soil	4.60±1.33	1.29±1.04	2.35±0.33	1.31±0.15	1.93±0.95
Drilling mud	141.71±64.80	47.20±7.93	39.36±13.38	11.81±3.09	15.02±10.20
Experimental III					
Top soil	8.09±3.94	2.79±0.83	0.64±0.64	1.22±0.54	3.42±2.73
Drilling mud	197.87±77.38	100.32±50.16	59.38±55.88	11.33±10.55	9.65±9.37
Sand Layer					
Control I	----------	----------	----------	----------	ND
Control II	----------	----------	----------	----------	0.35±0.60
Experimental I	----------	----------	----------	----------	0.24±0.21
Experimental II	----------	----------	----------	----------	0.77±0.54
Experimental III	----------	----------	----------	----------	ND

*ND= Not Detectable (0 mg/kg) (EPA Method SW-846 8015B, triplicate analyses of triplicate sets)

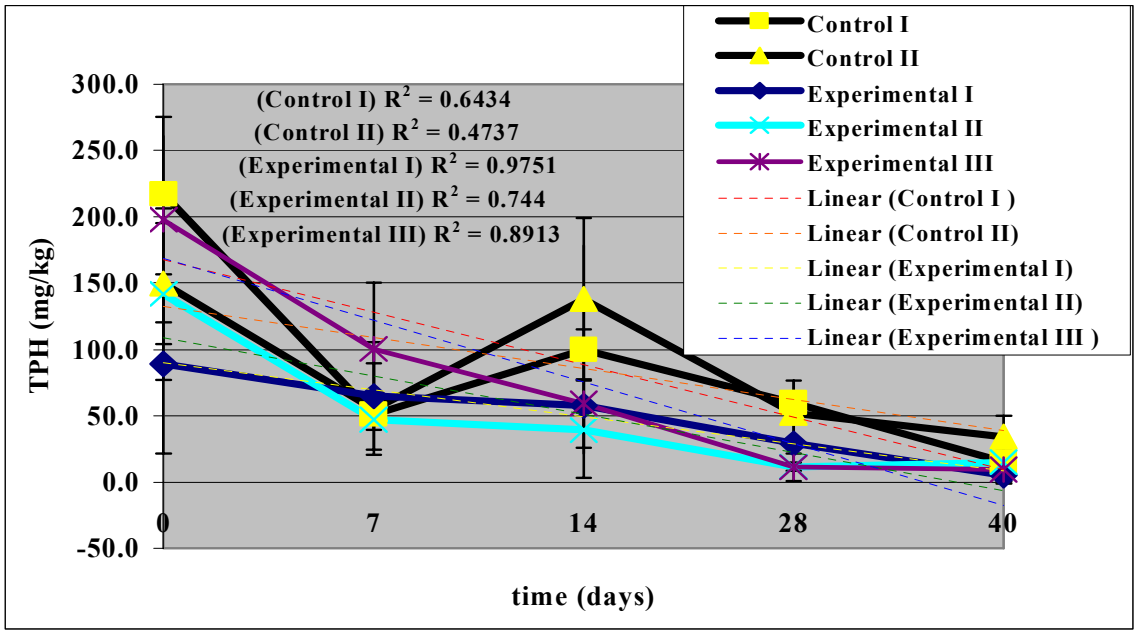

Figure 5.4: Total petroleum hydrocarbon trend of drilling mud:
Control vs. Experimental treatments-Microcosm study (P<0.05)
(EPA Method SW-846 8015B, triplicate analyses of triplicate sets)

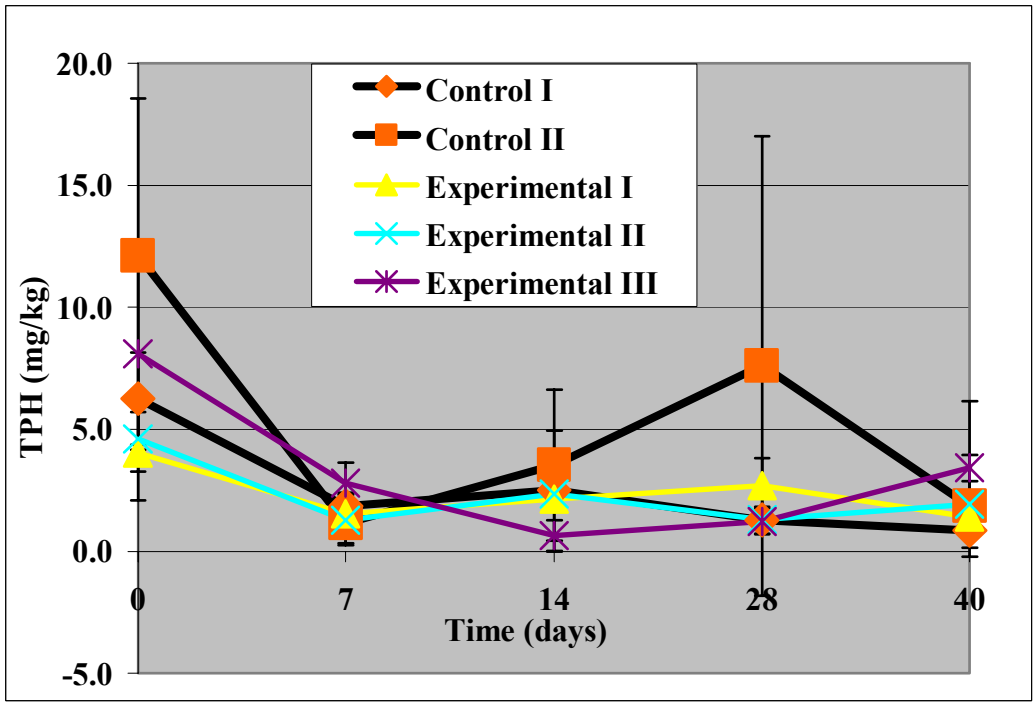

Figure 5.5: Total petroleum hydrocarbon trend of topsoil:
Control vs. Experimental treatments-Microcosm study
(EPA Method SW-846 8015B, triplicate analyses of triplicate sets)

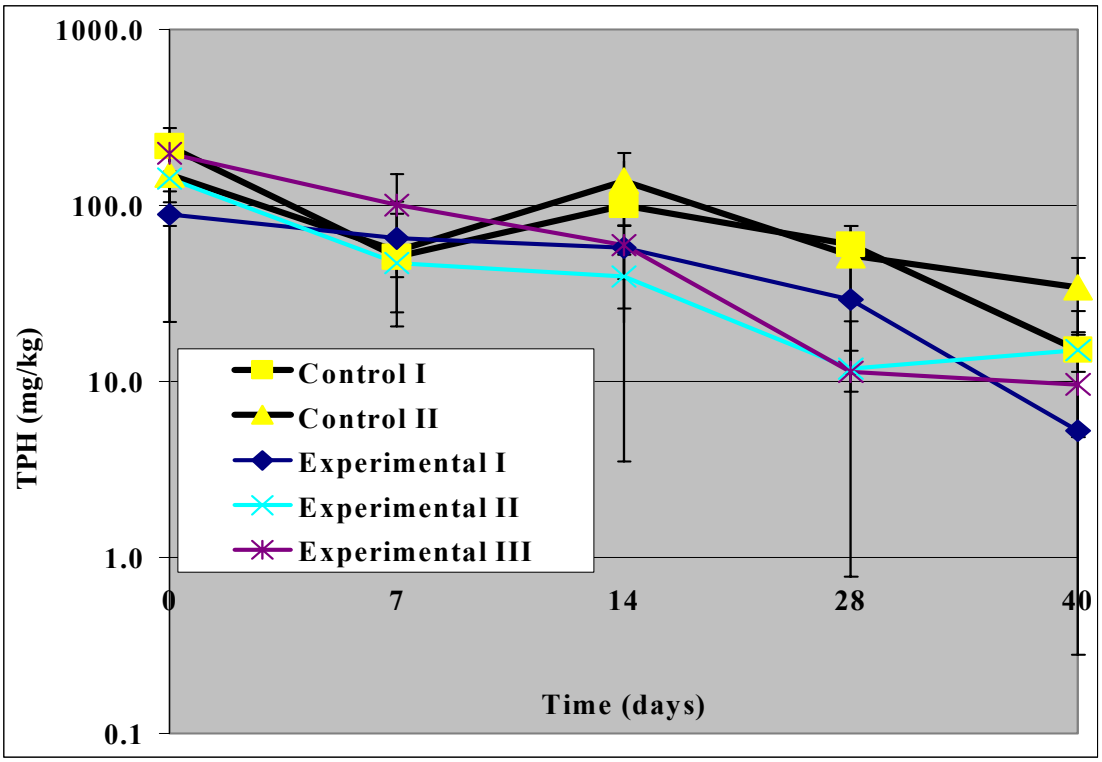

Figure 5.6: Logarithmic scale of drilling mud reduction:
Control vs. Experimental treatments-Microcosm study
(EPA Method SW-846 8015B, triplicate analyses of triplicate sets)

5.2.2 Polycyclic Aromatic Hydrocarbons Residuals: Phase Two Study

The following tables, 5.16-5.18, and figures, 5.7 and 5.8, show the polycyclic aromatic hydrocarbon profile of control and experimental drilling mud test beds for 0, 14, and 40d. The various components present in the initial drilling mud were phenol, naphthalene, 2-methylnaphthalene, acenaphthylene, acenaphthene, dibenzofuran, fluorene, phenanthrene, carbazole, fluoranthene, and benzo (a) anthracene. Net concentration of controls I and II at t=0d were 36.8±6.9 and 87.5±22.9 ug/kg, respectively. Experimental test beds I-III at t=0d had concentrations of 65.6±12.0, 158.2±54.4, 199.4±68.6 ug/kg, respectively. After 14d, control test bed samples had net concentrations of 129.6±89.4 and 173.9±53.7 ug/kg, respectively. The increase in PAH/phenol concentration for control test beds was due to the heterogeneity of the soil and the random sample collection during the experiment. Experimental test beds after 14d had concentrations of 61.4±29.3, 34.0±29.1, and 53.7±57.1 ug/kg, respectively. Upon completion of the experiment, control test beds had net concentrations of 26.1±17.5 and 25.6±13.4 ug/kg, respectively. Experimental test beds at 40d had concentrations of 20.1±19.7, 35.0±42.5, 44.4±39.0 ug/kg, respectively. The data indicates that more environmentally persistent components are present within the control drilling mud test beds than experimental (biologically treated) drilling mud. The experiment showed a 69.4-77.9% reduction in PAH and phenols for biologically treated drilling mud using an *in situ* remediation approach in a 40d period. Appendices C and D are the polycyclic aromatic hydrocarbon chromatograms from control and experimental drilling mud test beds, which includes saturate and aromatic profiles before and after treatment.

Table 5.16: Polycyclic aromatic hydrocarbon profile of control and experimental drilling mud (t=0d): Microcosm study

Drilling Mud Before Treatment	*CDM I*	*CDM II*	*EDM I*	*EDM II*	*EDM III*
Compound	*t*=0d	*t*=0d	*t*=0d	*t*=0d	*t*=0d
Phenol	1.2±0.6	2.7±0.6	4.7±0.2	9.2±1.7	14.3±9.9
2-chlorophenol	0	0	0	0	0
2-Methylphenol (o-cresol)	0	0	0	0	0
2-Methylphenol (p-cresol)	0	0	0	0	0
2,4-Dimethylphenol	0	0	0	0	0
2,4-Dichlorophenol	0	0	0	0	0
Naphthalene	0.6±0.3	1.1±0.4	2.4±0.1	3.8±1.4	4.3±0.4
4-Cl-3-methylphenol	0	0	0	0	0
2-Methylnaphthalene	0.8±0.4	1.4±0.2	2.6±0.5	4.9±3.2	6.3±1.6
2,4,6-Trichlorophenol	0	0	0	0	0
Acenaphthylene	1.3±1.2	4.3±1.3	0	0	0
Acenaphthene	4.7±3.7	8.5±0.7	9.1±4.3	27.4±0.3	28.7±1.2
Dibenzofuran	15.6±3.8	38.9±12.5	28.7±7.3	66.4±22.7	85.6±32.1
2,3,4,6-Tetrachlorophenol	0	0	0	0	0
Fluorene	3.7±2.2	11.2±4.2	4.4±1.2	11.4±8.6	19.5±10.6
Pentachlorophenol	0	0	0	0	0
2,4-Dinitrophenol	0	0	0	0	0
Phenanthrene	5.2±1.3	17.5±7.8	12.0±0.8	29.9±24.6	40.7±17.7
Anthracene	0	0	0	0	0
Carbazole	0.6±0.5	0	0	0	0
Fluoranthene	0	0	1.7±1.7	0	0
Benzo (a) Anthracene	3.2±4.3	2.1±3.6	0	0	0
Chrysene	0	0	0	0	0
Benzo (b) fluoranthene	0	0	0	0	0
Benzo (k) fluoranthene	0	0	0	0	0
Benzo (a) pyrene	0	0	0	0	0
Dibenzo (a,h) anthracene	0	0	0	0	0
Indeno (1,2,3-cd) pyrene	0	0	0	0	0
Total PAH (ug/kg)	35.6±6.3	84.8±22.3	60.9±11.8	149.1±52.7	185.1±58.7
Total Phenol (ug/kg)	1.2±0.6	2.7±0.6	4.7±0.2	9.2±1.7	14.3±9.9
Total PAH & Phenol (ug/kg)	36.8±6.9	87.5±22.9	65.6±12.0	158.2±54.4	199.4±68.6

*CDM=Control drilling mud treatment. EDM=Experimental drilling mud treatment.
(EPA Method SW-846 8270C, triplicate analyses of triplicate sets)

Table 5.17: Polycyclic aromatic hydrocarbon profile of control and experimental drilling mud (t=14d): Microcosm study

Biotreated Drilling Mud	*CDM I*	*CDM II*	*EDM I*	*EDM II*	*EDM III*
Compound	14d	14d	14d	14d	14d
Phenol	6.5±2.3	6.2±2.4	18.7±26.0	2.6±2.7	4.7±4.3
2-chlorophenol	0	0	0	0	0
2-Methylphenol (o-cresol)	0	0	0	0	0
2-Methylphenol (p-cresol)	0	0	0	0	0
2,4-Dimethylphenol	0	0	0	0	0
2,4-Dichlorophenol	0	0	0	0	0
Naphthalene	2.8±2.0	3.2±0.9	0.9±0.3	1.2±1.0	1.6±1.5
4-Cl-3-methylphenol	0	0	0	0	0
2-Methylnaphthalene	2.7±2.0	4.0±1.3	1.6±1.6	1.1±0.9	1.6±1.3
2,4,6-Trichlorophenol	0	0	0	0	0
Acenaphthylene	0	0	0	0	0
Acenaphthene	24.7±9.9	22.3±7.7	13.5±0.5	7.2±5.6	14.5±12.8
Dibenzofuran	57.8±44.5	78.0±23.8	15.8±3.1	13.7±12.7	19.6±23.3
2,3,4,6-Tetrachlorophenol	0	0	0	0	0
Fluorene	11.5±9.9	17.2±5.9	3.5±1.2	2.3±2.3	3.7±4.6
Pentachlorophenol	0	0	0	0	0
2,4-Dinitrophenol	0	0	0	0	0
Phenanthrene	23.5±19.5	34.8±11.9	6.7±1.9	5.4±4.5	8.0±9.7
Anthracene	0	0	0	0	0
Carbazole	0	0	0	0	0
Fluoranthene	0	8.1±4.2	0.7±1.2	0.6±1.0	0
Benzo (a) Anthracene	0	0	0	0	0
Chrysene	0	0	0	0	0
Benzo (b) fluoranthene	0	0	0	0	0
Benzo (k) fluoranthene	0	0	0	0	0
Benzo (a) pyrene	0	0	0	0	0
Dibenzo (a,h) anthracene	0	0	0	0	0
Indeno (1,2,3-cd) pyrene	0	0	0	0	0
Total PAH (ug/kg)	123.0±87.3	167.7±51.9	42.7±5.9	31.4±26.5	49.0±52.8
Total Phenol (ug/kg)	6.5±2.3	6.2±2.4	18.7±26.0	2.6±2.7	4.7±4.3
Total PAH & Phenol (ug/kg)	129.6±89.4	173.9±53.7	61.4±29.3	34.0±29.1	53.7±57.1

*CDM=Control drilling mud treatment. EDM=Experimental drilling mud treatment
(EPA Method SW-846 8270C, triplicate analyses of triplicate sets)

Table 5.18: Polycyclic aromatic hydrocarbon profile of control and experimental drilling mud (t=40d): Microcosm study

Biotreated Drilling Mud	*CDM I*	*CDM II*	*EDM I*	*EDM II*	*EDM III*
Compound	40d	40d	40d	40d	40d
Phenol	1.7±0.6	2.0±0.8	0	1.1±1.0	0
2-chlorophenol	0	0	0	0	0
2-Methylphenol (o-cresol)	0	0	0	0	0
2-Methylphenol (p-cresol)	0	0	0	0	0
2,4-Dimethylphenol	0	0	0	0	0
2,4-Dichlorophenol	0	0	0	0	0
Naphthalene	0.9±0.2	1.0±0.3	0	0.6±0.5	0
4-Cl-3-methylphenol	1.0±0.3	0.3±0.5	0	0	0
2-Methylnaphthalene	1.2±1.0	0.8±0.3	0	0.5±0.4	0
2,4,6-Trichlorophenol	0	0	0	0	0
Acenaphthylene	0	0	0	0	0
Acenaphthene	3.7±1.4	8.0±4.4	18.8±18.7	29.9±46.9	44.2±39.3
Dibenzofuran	11.4±11.1	7.5±5.0	0	2.4±2.1	0
2,3,4,6-Tetrachlorophenol	0	0	0	0	0
Fluorene	2.0±1.9	1.2±0.9	0	0.24±0.21	0
Pentachlorophenol	0	0	0	0	0
2,4-Dinitrophenol	0	0	0	0	0
Phenanthrene	3.0±2.1	3.4±1.9	1.4±1.0	0.28±0.24	0.2±0.4
Anthracene	0	0	0	0	0
Carbazole	0.3±0.5	0	0	0	0
Fluoranthene	1.1±0.5	1.5±0.5	0	0	0
Benzo (a) Anthracene	0	0	0	0	0
Chrysene	0	0	0	0	0
Benzo (b) fluoranthene	0	0	0	0	0
Benzo (k) fluoranthene	0	0	0	0	0
Benzo (a) pyrene	0	0	0	0	0
Dibenzo (a,h) anthracene	0	0	0	0	0
Indeno (1,2,3-cd) pyrene	0	0	0	0	0
Total PAH (ug/kg)	23.4±17.6	23.2±13.0	20.1±19.7	33.9±43.4	44.4±39.0
Total Phenol (ug/kg)	2.7±0.9	2.3±0.8	-	1.1±1.0	-
Total PAH & Phenol (ug/kg)	26.1±17.5	25.6±13.4	20.1±19.7	35.0±42.5	44.4±39.0

*CDM=Control drilling mud treatment. EDM=Experimental drilling mud treatment (EPA Method SW-846 8270C, triplicate analyses of triplicate sets)

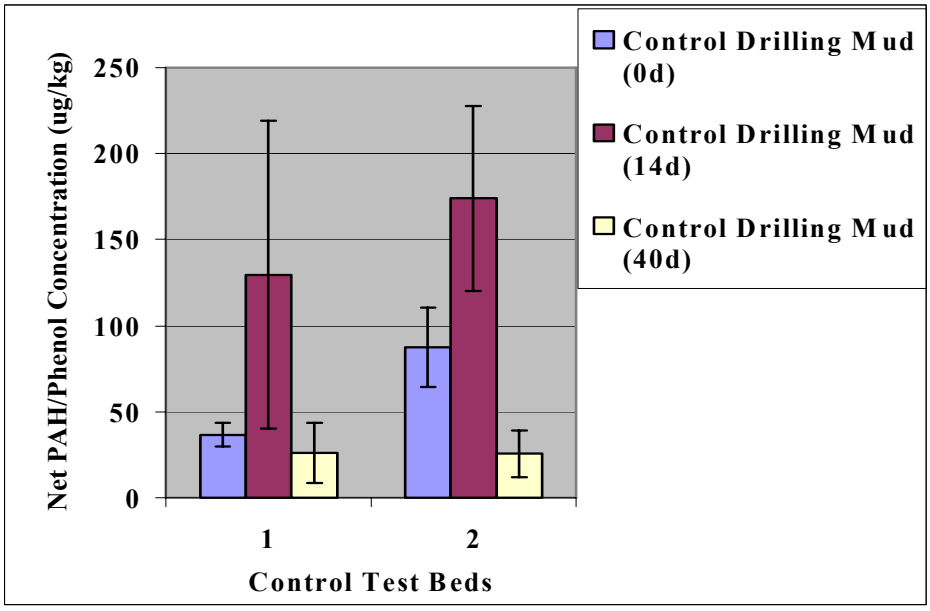

Figure 5.7: Polycyclic aromatic hydrocarbon trend: Control drilling mud-Microcosm study. (EPA Method SW-846 8270C, triplicate analyses of triplicate sets)

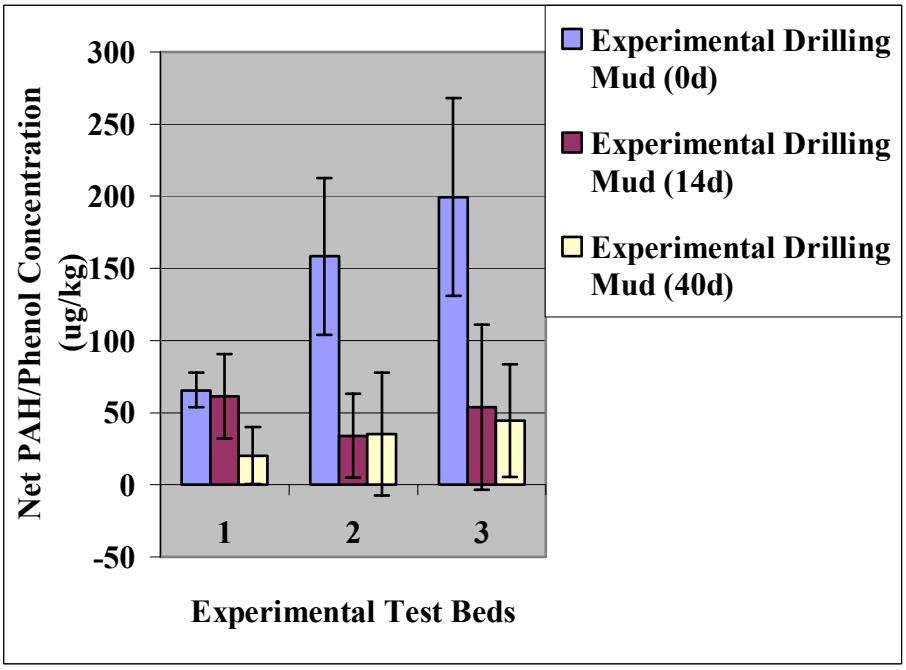

Figure 5.8: Polycyclic aromatic hydrocarbon trend: Experimental drilling mud-Microcosm study. (EPA Method SW-846 8270C, triplicate analyses of triplicate sets)

5.2.3 Residual Metals Analysis: Phase Two Study

Table 5.19 represents a metals profile for both control and experimental microcosms for the topsoil, drilling mud, and sand layers. The data indicates the greatest Pb levels were detected in the treated sand with 7.52±2.46 mg/kg in concentration. The following metals for the sand layer: sodium, potassium, calcium, magnesium, iron, manganese, zinc, and nickel, had a lower concentration compared to the drilling mud and top soil by a factor of ten largely due to the sand acting as a test layer. The purpose of the sand layer was to assess the extent of metal leachate from the soil/mud and that it simulated the underlying soil layer. Arsenic levels were found to contain 0.11 mg/kg in all control and experimental soil/drilling mud samples. In the previous metals analysis (Phase I), arsenic had been found at detection limit, which was 0.06 mg/kg or less. Iron complexes were largely detected in the control and experimental drilling mud. The zinc present in the drilling mud was three to four times higher in concentration compared to the topsoil (Analysis using DTPA-extractant). The topsoil had shown a decrease in zinc concentration after treatment (Analysis using HCl-extractant) (Page et al, 1982).

5.2.4 Soil Microbe and Nutrient Data: Phase Two Study

Figure 5.9 shows an interpolated assessment of microbial activity in the recycled water using a HYcheck^{TM} dipstick. Microbial bleedoff was first assessed at day 2 for both control and experimental setups and then was monitored on a weekly basis. There were erratic changes in microbial bleedoff for the controls. Fairly high microbial activity was imminent within the first 14 days of the experiment for all setups. Control I and Experimental III had a steady decline in microbial activity between days 28 and 40 from 10^6-10^3 CFU/ml. Experimental II had a plateau effect between days 21 and 40 at around

Table 5.19: Metals profile: Control and experimental topsoil, drilling mud, and sand layer-Microcosm study

Metal	*Control top soil (mg/kg)*	*Treated top soil (mg/kg)*	*Control drilling mud (mg/kg)*	*Treated drilling mud (mg/kg)*	*Control sand (mg/kg)*	*Treated sand (mg/kg)*
P	19.0±4.2	10.7±3.2	26.0±4.2	30.3±0.6	18.5±0.7	16.7±4.7
Na	980.0± 168.3	642.0± 54.5	536.0±7.1	669.7±93.4	79.5±0.7	122.3± 29.1
K	412.0± 137.2	312.7± 10.7	288.0±4.2	361.3±20.0	9.5±0.7	21.7±4.2
Ca	9412.0± 3650.1	7288.3± 62.3	5253.0± 561.4	5466.0± 525.4	236.5± 101.1	331.7± 47.9
Mg	567.5± 205.8	415.0± 25.4	350.0±41.0	372.7±18.3	20.0±5.7	29.0±1.7
Cu	3.19±0.46	2.88±0.19	4.86±0.41	5.16±0.37	1.40±0.11	2.24± 0.51
Fe	52.3±10.5	44.2±4.4	97.8±12.2	105.0±13.0	9.2±1.6	10.2±0.8
Mn	16.7±1.4	14.1±0.4	54.0±0.9	58.6±3.9	2.42±0.33	2.84± 0.42
Zn*	16.2±9.0	10.8±1.9	47.4±17.5	49.3±7.4	1.19±0.39	2.03± 0.55
As	0.11	0.11	0.11	0.11	0.11	0.11
Cd	0.06±0.03	0.04±0.01	0.17±0.06	0.15±0.02	0.04	0.05± 0.02
Ni	0.78±0.39	0.49±0.04	1.23±0.36	1.35±0.25	0.15±0.02	0.27± 0.27
Pb	0.31	0.31	0.98±0.95	0.31	4.91±2.00	7.52± 2.46
Zn**	4.98±5.85	0.84±0.15	37.4±25.1	29.3±8.8	2.38±0.59	4.29± 2.24

Reference: (Page et al, 1982), Controls: Triplicate analyses of duplicate sets, Experimental (treated sample): Triplicate analyses of triplicate sets. *DTPA Method. **HCl Method. Shaded value denotes significant change ($P<0.05$).

10^6-10^7 CFU/ml. A sharp decline in microbial activity took place for control II between days 35 and 40 from 10^6-10^1 CFU/ml. Experimental I was between 10^4-10^7 CFU/ml.

Figure 5.10 shows the nutrient trend of ammonia for experimental setups. Between days 14 and 21, a steady increase was noticed to approx. 50 mg/L and between

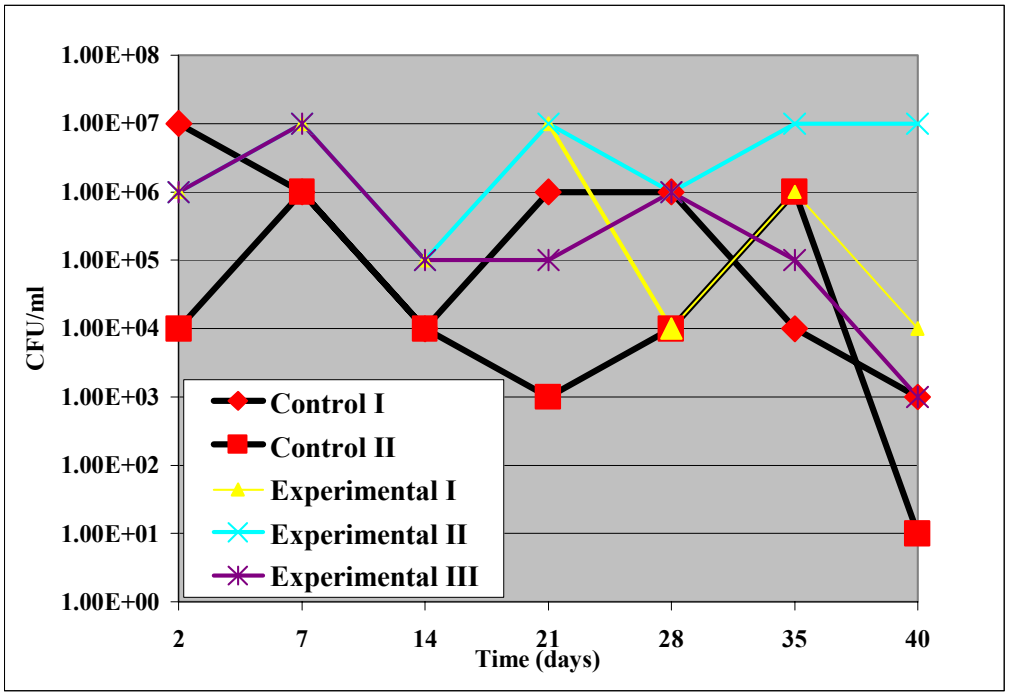

Figure 5.9: Variation in microbial activity of aqueous phase aliquots: Microcosm study (HYcheck^{TM})

days 25 and 40, a plateau effect took place at around 60-100 mg/L for all setups. Figure 5.11 shows the nutrient trend of phosphate for experimental setups. Between days 14 and 25, a slight increase was noticed to approx. 10 mg/L, a slight decrease for experimental I and II on day 29 to 40 mg/L, between days 29 and 37, a sharp increase in phosphate occurred to 18 mg/L and then decreased at day 40 to approx. 8 mg/L for all setups. Figure 5.12 shows the nutrient trend of nitrate for experimental setups. A steady increase in nitrate levels occurred between days 14 and 37 at around 20-60 mg/L and a sharp increase in nitrate levels occurred for experimental I by day 40 to 120 mg/L. The pH of the recycled water was also monitored during the course of the experiment. The control and experimental effluent had a pH from 7.0-7.5 and 7.0-8.0 over the 40 d period.

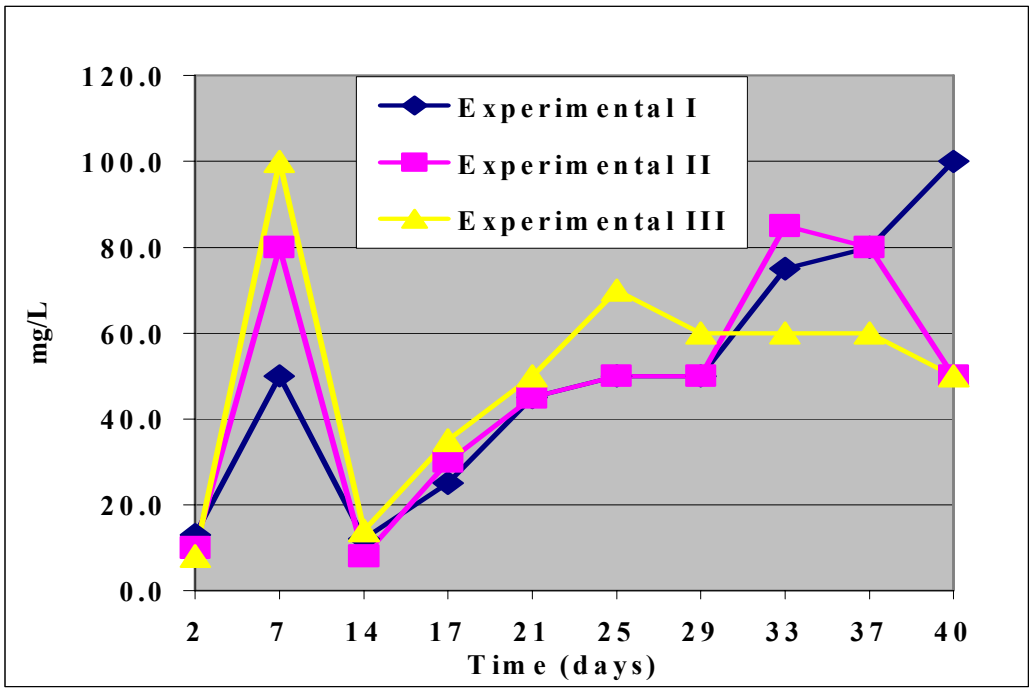

Figure 5.10: Variations in soil leachate residuals: Ammonia (mg/L)-
Microcosm study (CHEMets®)
Control I and II: Non-detectable, not listed on graph (0 mg/L)

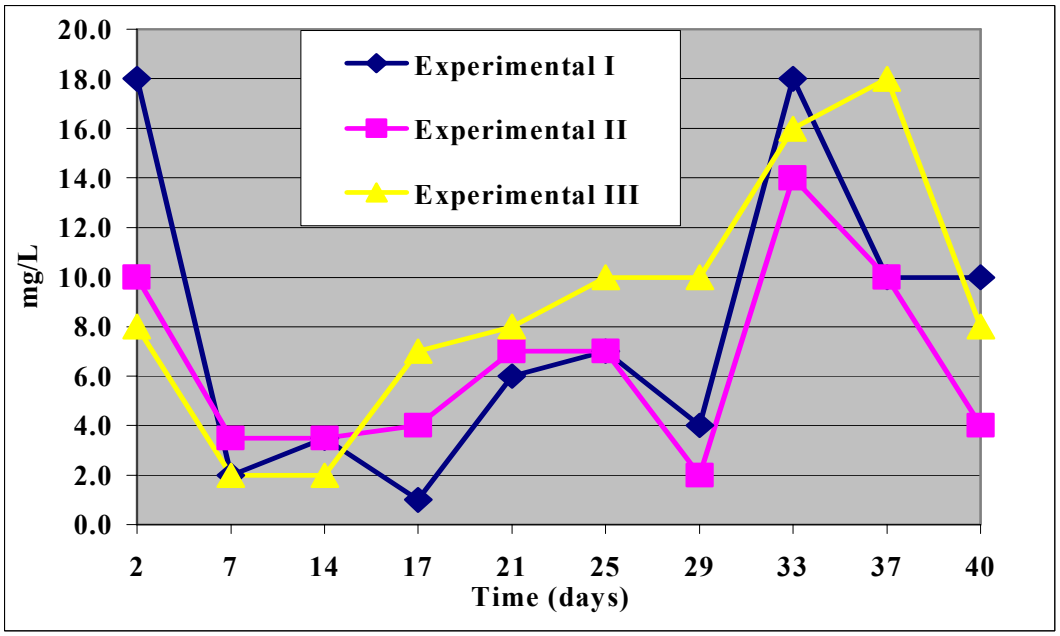

Figure 5.11: Variations in soil leachate residuals: Phosphate (mg/L)-
Microcosm study (CHEMets®)
Control I and II: Non-detectable, not listed on graph (0 mg/L)

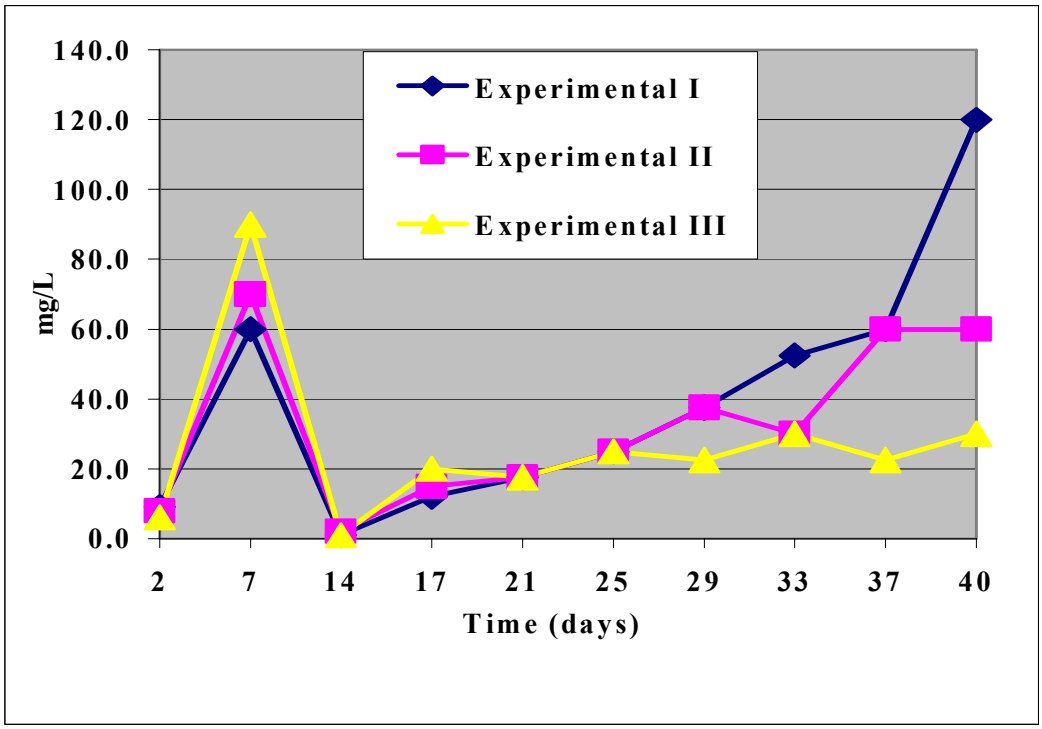

Figure 5.12: Variations in soil leachate residuals: Nitrate (mg/L)-
Microcosm study (CHEMets®)
Control I and II: Non-detectable, not listed on graph (0 mg/L)

5.2.5 Leachate Residual Analysis: Phase Two Study

Table 5.20 and Figure 5.13 shows the chemical oxygen demand profile for the control and experimental drilling mud upon completion of the 40d microcosm study. In this study, a significant leachable fraction was present for experimental I and III after 336h, which contained 29.0±12.7 and 59.5±2.1 mg/L, respectively. Experimental I and III trends were both consistent with each other, decreased after one week and then peaked at the final sampling point. The data suggests that the residual portion of drilling mud needs further remediation to obtain lower COD values. Aqueous samples from the Italian leachability test were preserved for a metals profile; data points were at the beginning and end points of the experiment at 2 and 336 hours.

Table 5.20: Chemical oxygen demand (COD) profile of control and experimental drilling mud: Microcosm study*

Time (hrs.)	*Control I (mg/L)*	*Control II (mg/L)*	*Experimental I (mg/L)*	*Experimental II (mg/L)*	*Experimental III (mg/L)*
2	3.5±2.1	15.5±3.5	5.0±2.8	18.0±2.8	1.5±2.1
8	12.0±2.8	17.5±2.1	11.5±3.5	21.5±7.8	2.5±0.7
24	8.0±2.8	2.5±3.5	10.5±2.1	21.5±2.1	7.5±6.4
48	15.0±5.7	0.5±0.7	13.0±1.4	19.5±0.7	6.5±0.7
72	14.0±1.4	0.5±0.7	1.0±0.0	22.5±4.9	18.0±9.9
102	13.5±0.7	2.0±0.0	8.0±4.2	23.0±0.0	5.5±0.7
164	17.5±0.7	4.5±2.1	4.5±3.5	6.5±0.7	12.0±7.1
336	20.0±2.8	15.0±0.0	29.0±12.7	18.0±2.8	59.5±2.1

*(Method 8000 for water, wastewater and seawater, duplicate analyses of duplicate sets)

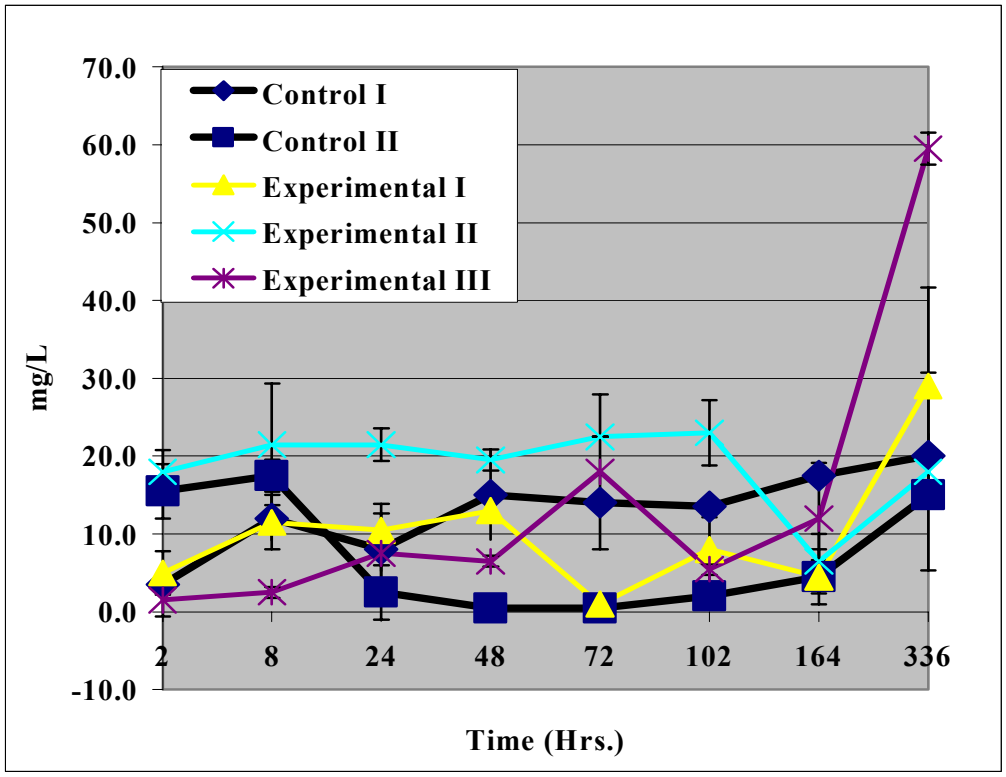

Figure 5.13: Variation in chemical oxygen demand (COD): Control vs. Experimental drilling mud-Microcosm study (Method 8000 for water, wastewater and seawater, duplicate analyses of duplicate sets)

Table 5.21: Metals speciation of leachable fraction: Control drilling mud Microcosm study*

Metal	Ba	Cr	Fe	Hg
	mg/L	mg/L	mg/L	mg/L
Control I 2h	0.092	<0.006	0.017	<0.0002
Control I 336h	0.112	<0.006	0.437	<0.0002
Control II 2h	0.150	<0.006	<0.006	<0.0002
Control II 336h	0.731	0.048	0.749	<0.0002
Italian Limit	1.000	0.050	----	0.0010
La DEQ Limit	2.000	0.110	----	0.0020

*All samples below Italian and La DEQ limits. Analyses not performed for As, Cd, Pb, Se, and Ag. (Methods 6010A and 7470A, U.S. EPA, 1983).

Metals speciation of leachate from control and experimental drilling mud was performed at 2 and 336 hours (Tables 5.21 and 5.22). Arsenic, cadmium, lead, selenium, and silver were not tested in the analysis. Baseline data for arsenic, cadmium, lead, selenium, and silver are noted in tables 5.11 and 5.12 (Phase one: Screening study). The leachate for the control drilling mud had indicated that all samples were below Italian and La DEQ limits. The leachate for the experimental drilling mud had indicated that all samples except experimental II at 2h were below the established limits. The leachate for the drilling mud shows a significant release of iron complexes after a 2-week period. The data suggests that the leachate of barium was possibly due to it complexation within the biomass of the soil and within the amended nutrients percolated through the system. The cause for a significant presence of mercury in the experimental II extract at 2h was unknown. Chromium and mercury are not of concern for the leachable fraction of the soil. Upper limits of detection for Ba, Cr, Fe, and Hg were 1.07, 1.08, 1.01, and 0.0052 mg/L, respectively. Lower limits of detection were 0.85 mg/L for Ba, Cr, and Fe, and 0.0043 mg/L for Hg (Analytical and Environmental Testing, Inc., 2001).

Table 5.22: Metals speciation of leachable fraction: Experimental drilling mud Microcosm study*

Metal	Ba	Cr	Fe	Hg
	mg/L	mg/L	mg/L	mg/L
Exp I 2h	0.131	<0.006	<0.006	<0.0002
Exp I 336h	0.186	<0.006	0.696	<0.0002
Exp II 2h	0.121	<0.006	<0.006	0.0025
Exp II 336h	0.141	<0.006	0.726	<0.0002
Exp III 2 h	0.143	<0.006	0.028	<0.0002
Exp III 336 h	0.198	<0.006	1.27	<0.0002
Italian Limit	1.000	0.050	----	0.0010
La DEQ Limit	2.000	0.110	----	0.0020

*White denotes less than (<), shaded denotes excedence (P<0.05).
Analyses not performed for As, Cd, Pb, Se, and Ag.
Exp=Experimental drilling mud.
(Methods 6010A and 7470A, U.S. EPA, 1983).

5.2.6 Residual Toxicity Analysis of Leachable Fractions: Phase Two Study

Each treatment had a positive effect on the luminescent bacteria, *Vibrio fischeri* (Figure 5.14). Control and experimental treatments indicated that with increasing concentration, positive effects took place for the dilutions that ranged from 5.625 to 45% concentrated solution. Sample leachate was preserved at days 14, 24, 35, and 40 from control and experimental microcosms and an average 15 min. % effect was graphed out.

The percent of affected bacteria ranged from –0.27 to –4.46% in the 15-minute interval for control and experimental treatments (5.625% solution). For the next dilution (11.25% solution), the percent of effected bacteria ranged from –1.42 to –10.33% in the 15-minute interval for control and experimental treatments. For the next dilution (22.5% solution), the percent of effected bacteria ranged from –3.45 to –14.13% in the 15-minute interval for control and experimental treatments. For the most concentrated dilution (45.0% solution), the percent of effected bacteria ranged from –1.91 to –14.71% in the 15-minute interval for the various treatments.

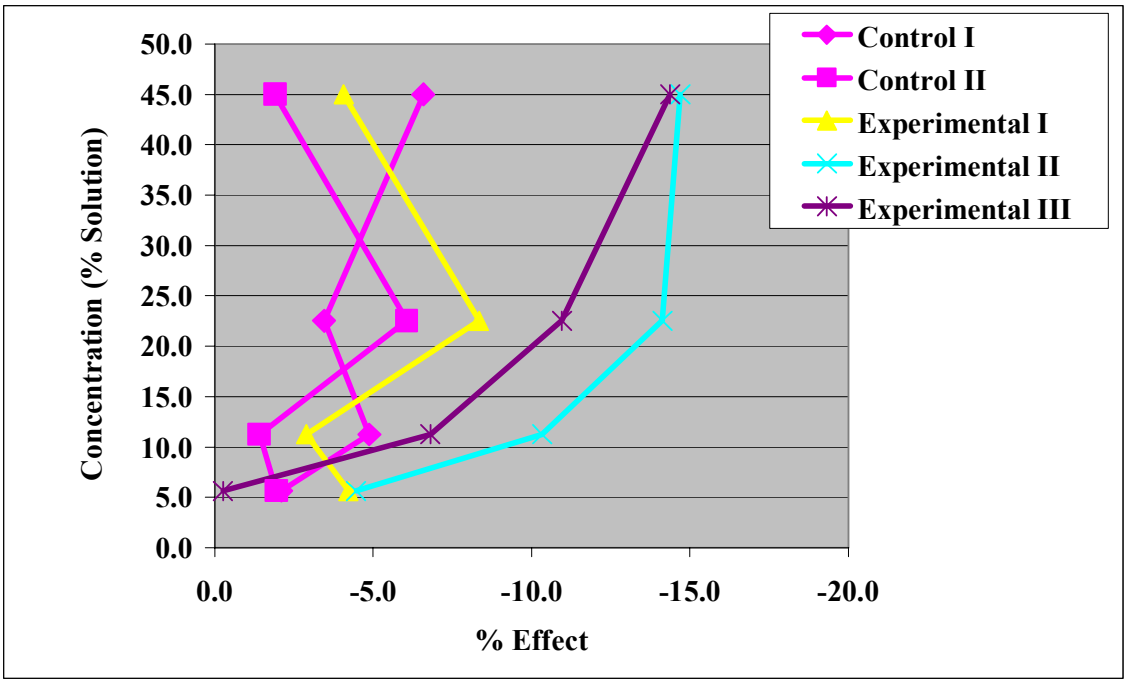

Figure 5.14: % Effects at 15 min. time interval for aqueous phase of control and experimental microcosms (Microtox®) ($P<0.05$)

The data indicated that toxicity was initially observed in the first 5 minutes of analysis (not reported). The experimental treatments had a more marked positive effect on bacteria than control treatments ($P<0.05$). The positive effect observed on the experimental extracts indicated that the bacteria were capable of withstanding exposure from samples with increasing concentration (% solution).

5.2.7 General Discussion of Phase Two Data

This experiment was performed to determine if an *in situ* bioremediation system using bioplug technology would be effective for the Italian site. The significance of the data shows that the addition of nutrient amendments for experimental setups enhanced the kinetic rate of microbial degradation. The reduction of total petroleum hydrocarbons within the controls represented natural attenuation, which was the continuous aeration and flow of recycled water through the system with no addition of amended nutrients. A

significant reduction had taken place in the drilling mud over the 40d period using bioplug technology. At the Italian site, to remediate the entire landscape will take years to complete, however, it is the most widely chosen and cost-effective method compared to land filling.

Microbial bleedoff was monitored to compare the difference of adding microflora (Experimental) versus microbes already present in the soil (Control). Nutrients were added to the recycled water for experimental setups, which flowed through the PVC pipe by gravity and exited out into the soil. The rate of adding nutrients into the system was assessed within the first 14 d of the experiment. Nutrients were added every 4 d to assure continuous abundance. Approximately 2 g/L of ammonium nitrate and 0.2 g/L potassium phosphate monobasic were added accordingly. Decline in microbial activity at day 40 was due to the conclusion of the experiment and availability of nutrients. After addition of nutrients, ammonia and nitrate were approximately 150 mg/L and phosphate was approximately 80 mg/L. Nutrient levels were also monitored for the controls where no nutrients were added and obtained non-detectable limits during the course of the experiment. Nutrient levels were determined using a CHEMet® colorimetric kit.

The data indicates that there was not a large significant difference in metal concentration of soils from control and experimental microcosms. This may be due to several reasons: (i) Treating the soil *in situ* means the soil was not homogenized (mixed), and (ii) The brevity of the experiment. All metal concentrations present in the soil/drilling mud/sand layers were well below Italian and La DEQ soil standards, which suggested that a chelating agent would not be required when treating the soil at the Italian site.

Sample leachate was preserved at days 14, 24, 35, and 40 from control and experimental microcosms, which were analyzed for residual toxicity. The purpose of analyzing residual toxicity was to determine during the remediation process the fate of toxicity and any observed effect on the marine species *Vibrio fischeri*; to what extent the leachate went from the solid to aqueous phase from the use of recycled water and amended nutrients used in the *in situ* bioremediation system. The control microcosms had indicated that an observed effect had taken place to the marine species from exposure to the aqueous aliquots obtained over the 40d period and a positive effect on bacteria had taken place (Negative % effect) over time for two out the three experimental microcosms. Overall, this experiment has shown that two out of three experimental microcosm test beds had exhibited a statistically significant decline in total petroleum hydrocarbons.

6. CONCLUSION

6.1 Summary of Findings

The hypothesis of the microcosm experiment was that drilling muds could be remediated using an *in situ* approach. Composite samples of drilling mud and topsoil were randomly chosen at days 0, 7, 14, 28, and 40 for analysis for both control and experimental setups. Over the 40d period, the data indicated that a significant reduction in total petroleum hydrocarbons had taken place in both control and experimental setups. The reduction in the controls was largely due to natural attenuation and experimental setups due to enhanced kinetic rate. It is suggested that the most degradation would take place between the bioplug configurations; however, it would eventually permeate throughout the entire setup. The drilling mud and topsoil layers which didn't get direct saturation of the effluent was gently applied onto the top surface 1-2x a week for an equal distribution among the soil. An indicator of the efficiency of biodegradation was from a total petroleum hydrocarbon assessment. The total petroleum hydrocarbon and PAH/Phenol data obtained for this experiment were not statistically identical largely due to the low concentrations present in the mud, which comprises of a light petroleum mixture. Both the screening study and *in situ* treatment resulted in effective reductions of drilling mud, however the latter is a preferred method largely because excavation and continuous mixing would require a lot of funding for treatment of the site. In the literature review, recall that drilling muds with low pH's will exhibit dermal and inhalation toxicity and CNS depression. The drilling mud in the treatability study had 7-8 for its pH. This was due to the buffering of the system, adding nutrient amendments to the soil, which included ammonium nitrate and KH_2PO_4 (monobasic) to control pH.

6.2 Meeting International Standards for *In situ* Bioremediation Approaches

The importance of performing a leachability experiment was to determine the chemical oxygen demand and metals profile of the leachable fraction of the drilling mud. If a high chemical oxygen demand was present, >30 mg/L, then further treatment is necessary to meet international standards. Another interpretation of a leachability experiment is, "How clean is clean for the Italian soil?" The data obtained for the drilling mud that had undergone remediation in the 40d microcosm study indicated that further remediation was required to decrease the chemical oxygen demand. The data also suggests that the experimentally treated drilling mud is more permeable than the control drilling mud, resulting in more porous space. When the soil is more permeable, water, microbes, and nutrients can percolate around the particles more readily. Another important variable when treating the drilling mud/soil was to obtain a metals profile to see what metals are persistent and which had degraded over time. When obtaining data for topsoil, drilling mud, and effluent from the biodegradation and 14d leachability experiments, the metals that had been tested are for priority metals. The metals profile for the topsoil and drilling mud indicated that levels were well below Italian and La DEQ standards. The metals profile for the leachable fraction of the control and experimental drilling mud indicated that Cd and Se had exceeded Italian and La DEQ limits. This indicates that special care should be taken when undergoing the remediation system at the Italian site. The drilling mud should be treated for as much time as possible to prevent the possible leachating of heavy metals into the groundwater. An important environmental chemistry concept when treating the soil is the presence of the carbon cycle and that it is a dynamic environment where the fate of toxicity moves and changes.

6.3 Suggested Analytical Protocols for International Applications

A major problem that was noted in the Italian protocol for the 14d leachability experiment was that the sample was placed in the reactor vessel in a spherical shape. When you do this, you are essentially minimizing the surface area. When the sample is placed into the enclosed reactor vessel undergoing saturation, the leachable fraction of the soil is taking place on the outer portion, where the water is going around the sample. The inner portion of the sample contains most of the contaminants of concern. A suggestion for improving the Italian protocol is to try doing a suspended solid test where the soil is broken up into pieces in an aqueous suspension or you can flatten out the soil so the leachable fraction occurs within the entire sample. When doing this, the chemical oxygen demand measurements should differ when executing this test.

Acceptable metal concentrations and contaminant levels for soil, drilling mud, and aqueous aliquots obtained at various time intervals should be based on the following variables: (i) The scale of remediation needed for the site and (ii) The available funds. A recommended approach to determining the scale of how long the system should run should be based on a cost-benefit analysis; how much the Italian government can fund in remediating the impoundments. A cost-benefit analysis and risk assessment can help determine to what levels are suitable for the agricultural landscape and prevention of toxicity of subsurface soils and ground water. The Italian limits that are established for the leachate of metals/hydrocarbons should be a recommended approach for this project. The Italian limits established suggest that they would want to use the landscape for further development/productivity.

6.4 Recommendations for Future Work

Future work needed for this project is to acquire a permit for installation of the bioplugs at Galliano, Sicily. The bioremediation process will have to be monitored by Italian contractors and continuous sampling must take place. Further research is needed for petroleum contaminated soils from Syracuse, Sicily. The bioplug technology utilized in the laboratory will be tested on the Syracuse sample. However, when considering the treatment of the soils, the pH must be considered. When tested, the pH of the soil was 12.0, which is highly basic for remediation unless a microorganism is selected which can handle high pH levels. A direct acidification may be necessary for proper treatment of the soil.

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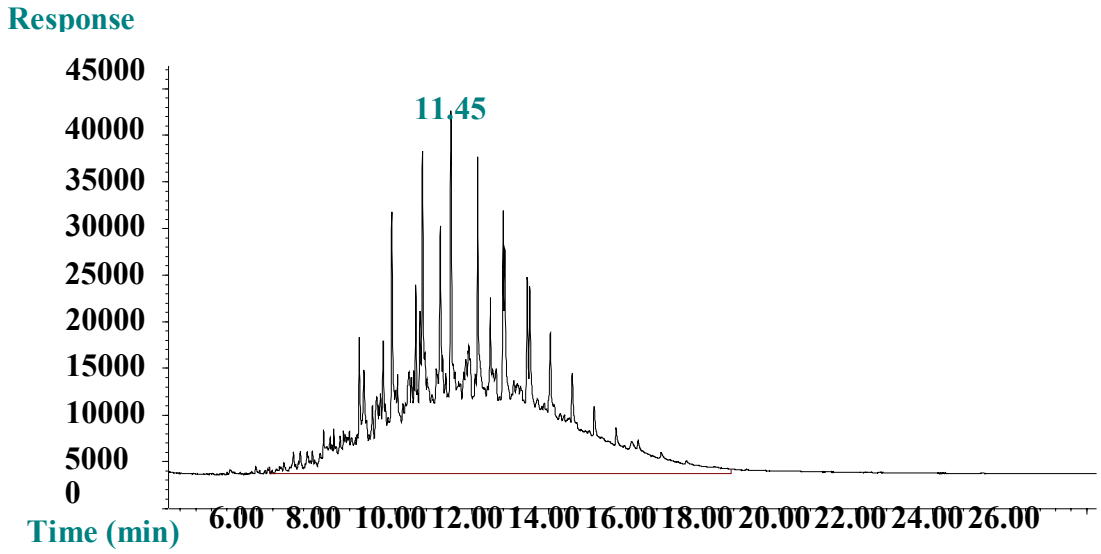
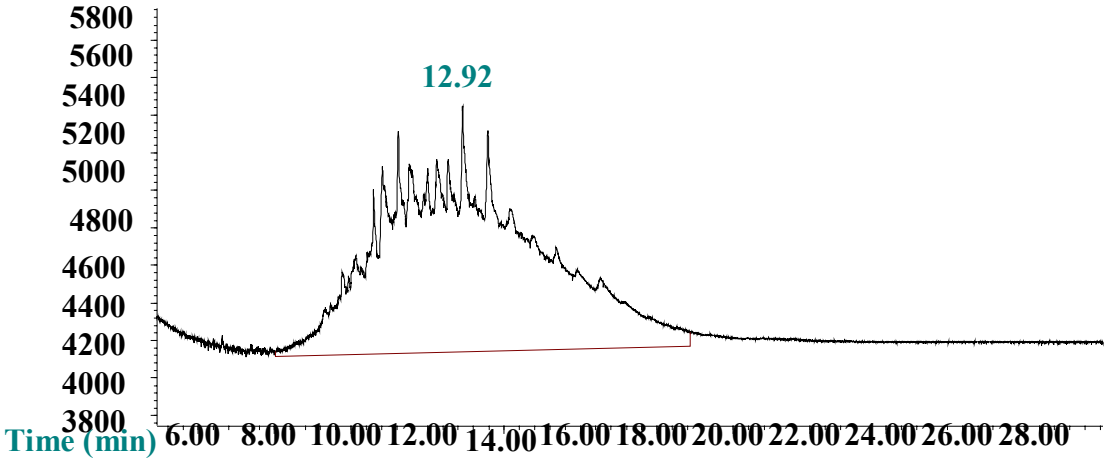
APPENDICES

A. Total Petroleum Hydrocarbon Chromatograms: Microcosm Study

1. Control Drilling Mud (t=0d)

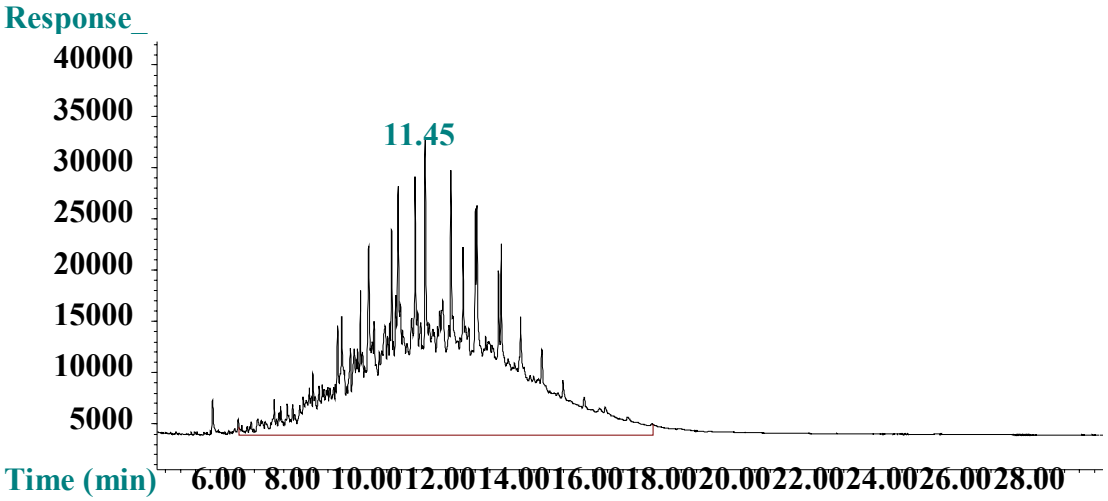


2. Control Drilling Mud (t=40d)

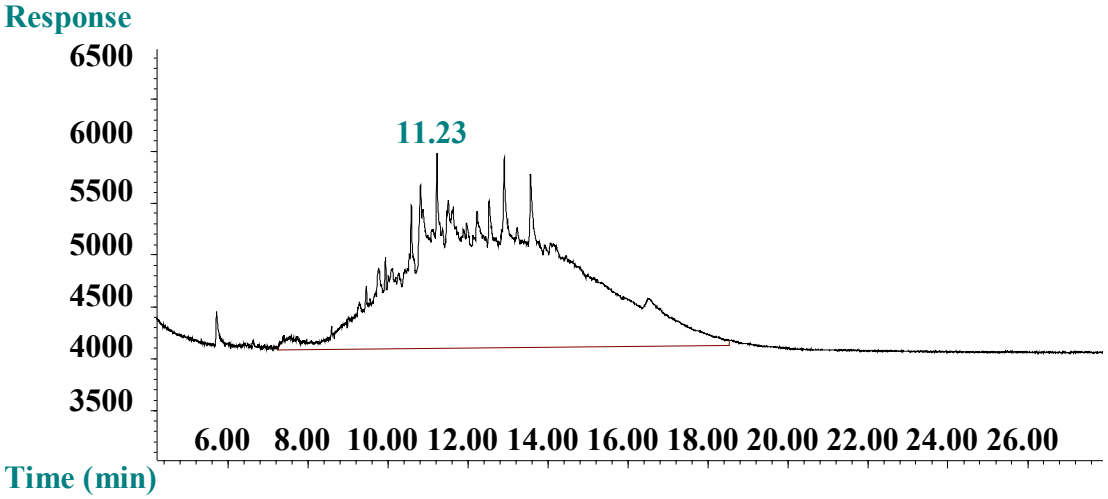


B. Total Petroleum Hydrocarbon Chromatograms: Microcosm Study

1. Experimental Drilling Mud (t=0d)

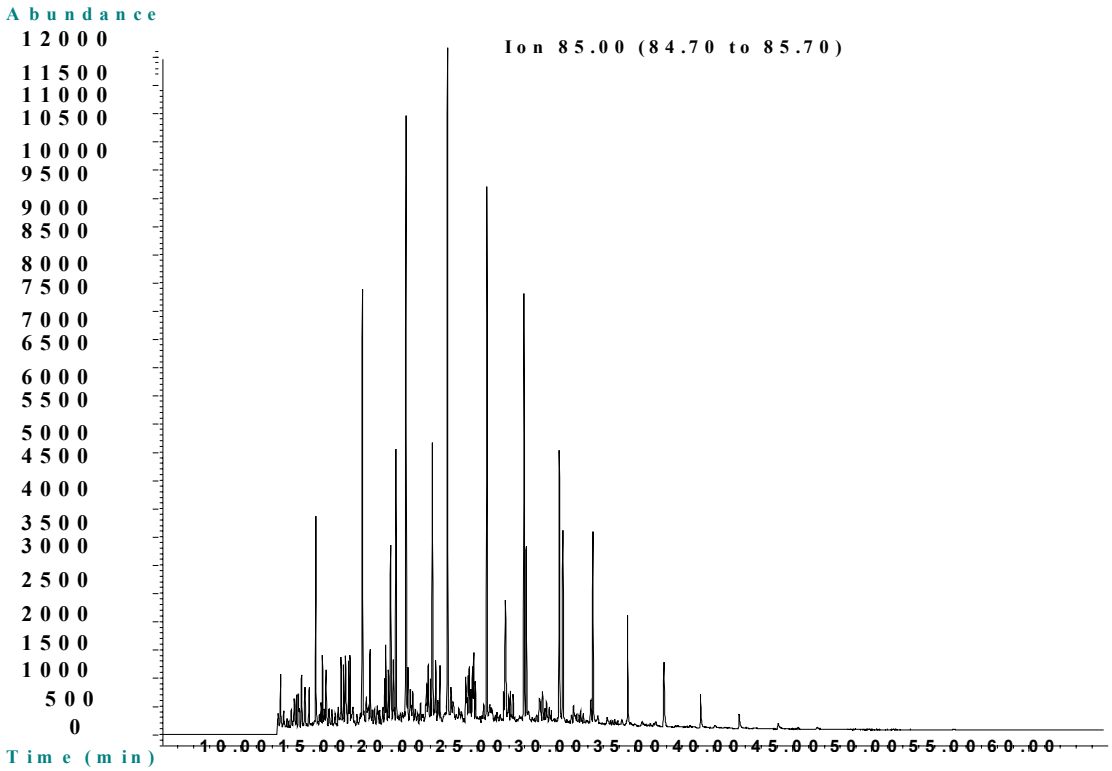


2. Experimental Drilling Mud (t=40d)

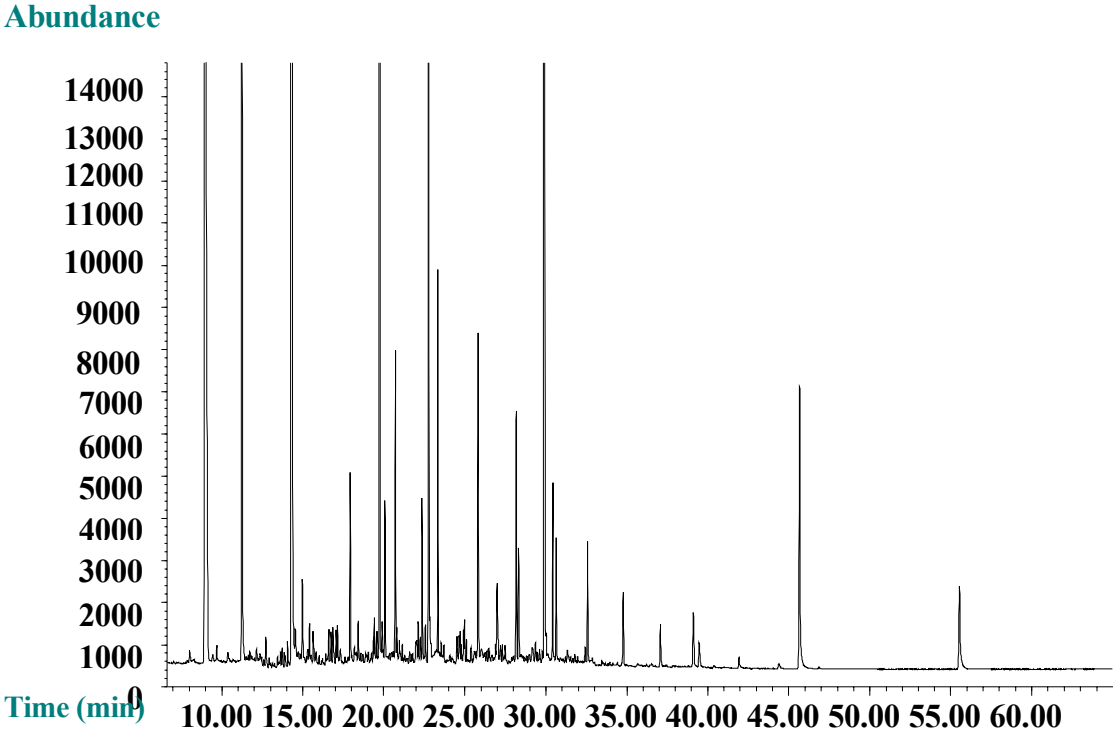


C. Polycyclic Aromatic Hydrocarbon Chromatograms: Microcosm Study

1. Control Drilling Mud at Ion 85

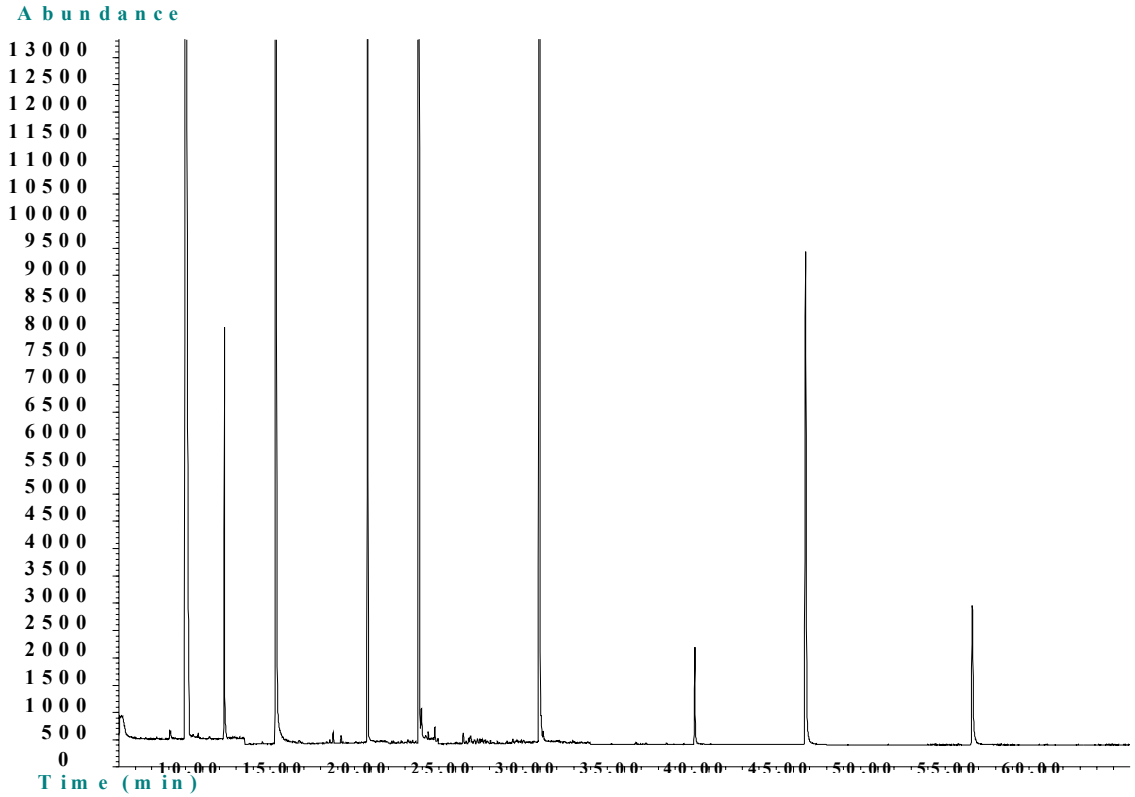


2. Total Ion Chromatogram of Drilling Mud Before Treatment

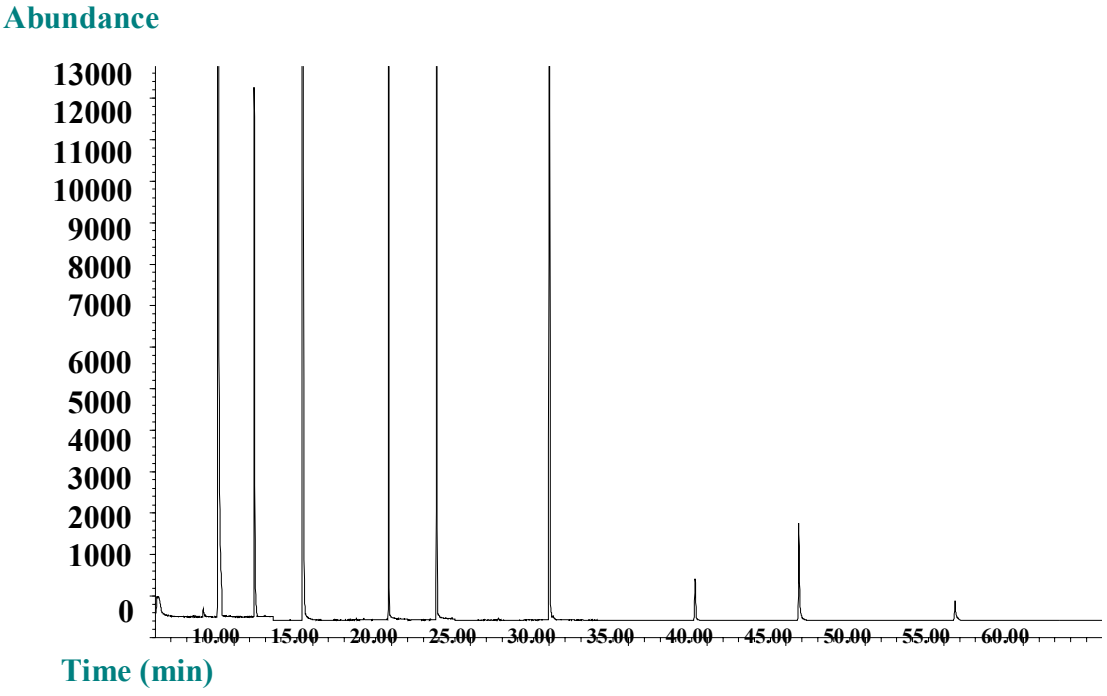


D. Polycyclic Aromatic Hydrocarbon Chromatograms: Microcosm Study

1. Control Drilling Mud (t=40d)



2. Experimental Drilling Mud (t=40d)



VITA

Jason Allan McDonald was born in Valley Stream, New York, on July 28, 1978. He is the oldest son of Grace McDonald of Elmont, New York, and has two sisters, Crystal and Heather. He graduated June 21, 1996 from Sewanhaka High School in Floral Park, New York. On May 21, 2000, he received his bachelor of science degree in environmental studies with minors in physics and philosophy from St. John's University in Jamaica, New York. He was offered an environmental studies internship at Brookhaven National Laboratory in Upton, NY in January of 2000 and continued his research until August of 2000. He was offered a graduate research assistantship by the Department of Environmental Studies, Louisiana State University, and began his graduate studies in environmental toxicology in August of 2000. Mr. McDonald is currently a candidate for a master of science degree in environmental sciences to be awarded on December 21, 2001.